Nos. 2014-1276, -1278

United States Court of Appeals for the Federal Circuit

MOMENTA PHARMACEUTICALS, INC.
AND SANDOZ INC.,

Plaintiffs-Appellants,

v.

AMPHASTAR PHARMACEUTICALS, INC.,
INTERNATIONAL MEDICATION SYSTEMS, LTD.,
ACTAVIS, INC. AND ACTAVIS PHARMA, INC.
(formerly known as Watson Pharma, Inc.),

Defendants-Appellees.

Appeals from the United States District Court for the District of Massachusetts in No. 1:11-cv-11681-NMG, Judge Nathaniel M. Gorton.

NON-CONFIDENTIAL BRIEF FOR PLAINTIFFS-APPELLANTS MOMENTA PHARMACEUTICALS, INC. AND SANDOZ INC.

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CERTIFICATE OF INTEREST

Counsel for plaintiffs-appellants Momenta Pharmaceuticals, Inc. and Sandoz Inc. certifies the following:

1. The full name of every party or amicus represented by me is:

Momenta Pharmaceuticals, Inc. and Sandoz Inc.

2. The name of the real party in interest (if the party named in the caption is not the real party in interest) represented by me is:

N/A

3. All parent corporations and any publicly held companies that own 10% or more of the stock of the party or amicus curiae represented by me are:

Momenta Pharmaceuticals, Inc.: BlackRock, Inc.
Sandoz Inc.: Novartis AG

4. The names of all law firms and the partners or associates that appeared for the party or amicus now represented by me in the trial court or are expected to appear in this court are:

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Dated: June 27, 2014 s/ Deanne E. Maynard

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CONFIDENTIAL MATERIAL

Materials that were made confidential pursuant to the protective order have been redacted from the non-confidential version of the brief. These materials include confidential business information from documents and exhibits filed in the district court.

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STATEMENT OF RELATED CASES

A preliminary injunction entered in this case previously was appealed to this Court. *Momenta Pharmaceuticals, Inc. v. Amphastar Pharmaceuticals, Inc.*, Nos. 2012-1062, -1103, -1104, 686 F.3d 1348 (Fed. Cir. Aug. 3, 2012) (Rader, C.J., Dyk, Moore, J.J.). The Court's decision in this case directly may affect or be affected by the Court's decision in *Momenta Pharmaceuticals, Inc. v. Teva Pharmaceuticals USA, Inc.*, Nos. 2014-1274, -1277 (Fed. Cir.), which also is pending on appeal from a final judgment. The *Amphastar* and *Teva* cases were heard before the same district court. Certain proceedings in the two cases were coordinated, but the cases were not formally consolidated. Counsel for plaintiffs-appellants are unaware of any other pending case that directly will affect or be affected by this Court's decision.

JURISDICTIONAL STATEMENT

The district court had jurisdiction under 28 U.S.C. §§ 1331 and 1338. It entered final judgment on January 24, 2014. Plaintiffs-appellants Momenta Pharmaceuticals, Inc. and Sandoz Inc. (collectively, "Momenta") appealed on January 30, 2014. This Court has jurisdiction under 28 U.S.C. § 1295(a).

STATEMENT OF THE ISSUES

1. Whether use by defendants (collectively, "Amphastar") of Momenta's patented methods for commercial manufacturing purposes in direct competition with Momenta is outside the safe harbor of 35 U.S.C. § 271(e)(1) because Amphastar's infringing conduct is not "solely for uses reasonably related to the development and submission of information under" the Federal Food, Drug, and Cosmetic Act ("FDCA"), where Amphastar's commercial manufacturing records are not submitted under the FDCA but merely maintained by Amphastar.

2. Whether, even if Amphastar's use of Momenta's patented invention is protected by Section 271(e)(1), Amphastar separately infringes under 35 U.S.C. § 271(g) because it offers to sell and sells enoxaparin that was manufactured using Momenta's claimed methods, and that separately infringing conduct does not fall within the plain terms of Section 271(e)(1) because those sales activities are not "solely for uses reasonably related to the development and submission of information under" the FDCA.

3. Whether Momenta should have been permitted to amend its infringement contentions to accuse two additional infringing Amphastar procedures because those procedures are separate, unprotected infringements.

INTRODUCTION

This appeal concerns the interpretation of two statutory provisions that are crucial to defining the scope of patent infringement in the context of pharmaceuticals: (1) the safe harbor provision in the Drug Price Competition and Patent Term Restoration Act ("Hatch-Waxman Act"), 35 U.S.C. § 271(e)(1), and (2) the infringement provision of 35 U.S.C. § 271(g). The text of these provisions governs. Yet the district court's interpretation of both provisions diverges from their statutory text. This appeal provides an opportunity for this Court to clarify the proper scope of the Section 271(e)(1) safe harbor and Section 271(g).

Momenta and Amphastar compete in the manufacture and sale of enoxaparin, a generic version of the branded drug Lovenox®. Momenta developed a process that can be used as part of enoxaparin manufacturing to control the quality and reduce the variability of the final product. It is undisputed on this record that Amphastar uses Momenta's patented methods. Even though Momenta's patent is fully in force, Amphastar uses it, without license, to manufacture enoxaparin for commercial purposes. That use infringes under Section 271(a). Amphastar then offers to sell and sells its enoxaparin made using

Mometa's patented process. That sales activity is a separate act of infringement, and it infringes under Section 271(g). Amphastar has made hundreds of millions of dollars from these sales, in direct competition with Momenta, without paying Momenta anything for use of its patented invention. A13050-A13073; A13288-A13300.

This infringing conduct is not protected by the Section 271(e)(1) safe harbor. As this Court held in the preliminary-injunction appeal in this case, the plain language of Section 271(e)(1) governs whether Amphastar's conduct is exempted from patent infringement. *Momenta Pharm., Inc. v. Amphastar Pharm., Inc.*, 686 F.3d 1348, 1353-54 (Fed. Cir. 2012) ("*Momenta I*"). Momenta agrees that is the correct legal question; indeed, Momenta agrees with much of the legal reasoning in *Momenta I*.

Section 271(e)(1) exempts otherwise-infringing conduct only if the conduct is "*solely* for uses *reasonably related* to the development and *submission* of information *under* a Federal law," here, the FDCA. 35 U.S.C. § 271(e)(1) (emphasis added). The safe harbor "allows competitors, prior to the expiration of a patent, to engage in otherwise infringing activities necessary to obtain regulatory approval" from the Food and Drug Administration ("FDA") of proposed commercial activity. *Eli Lilly & Co. v. Medtronic, Inc.*, 496 U.S. 661, 671 (1990). The safe harbor thus permits the approval process to occur during the patent term

so that the drug may be brought to market when the patent expires. In addition, as *Momenta I* recognized, the safe harbor protects certain post-approval conduct—*but only if* that conduct falls within the plain terms of Section 271(e)(1). 686 F.3d at 1358-59. The plain terms of Section 271(e)(1) do not permit competitors to use the patented invention to engage in the commercial activity itself during the life of the patent.

The district court nevertheless held Amphastar's otherwise-infringing commercial use exempt from infringement because FDA regulations require Amphastar to maintain records documenting each step of its manufacturing process—even though the records were not (and were never intended to be) "submi[tted] under" the FDCA. That holding cannot be squared with the statutory text.

"Submitting" information to the FDA and "maintaining" records for possible FDA inspection are distinct activities, and the text of the statutory scheme consistently and repeatedly uses those words to cover different conduct. The FDCA's textual distinction between information that must be "submitted" and records that must be "maintained" is crucial to the safe harbor's scope. Activities related to the development and submission of regulatory applications are precisely what the safe harbor was intended to protect. Conversely, a manufacturer's use of a patented invention during the ordinary commercial manufacture of its product is

not "reasonably related," much less "solely" so, to any "submission . . . under" the FDCA.

The district court rejected these arguments, believing itself bound by *Momenta I*'s suggestion that the mere maintenance of records may be considered a submission. But the district court should have considered the issue anew in light of the present, more complete record. The statutory distinction between "submission" and "maintenance" was not briefed to this Court in *Momenta I*, which was an expedited interlocutory appeal on a limited record. It is well established that "[a]n appellate court's preliminary injunction opinion has no conclusive bearing at the trial on the merits and is not binding on a subsequent panel." *Glaxo Grp. Ltd. v. Apotex, Inc.*, 376 F.3d 1339, 1346 (Fed. Cir. 2004).

Moreover, the record now establishes that, contrary to Amphastar's primary argument in *Momenta I*, the FDA does not mandate Amphastar's use of Momenta's patented method. Rather, in its ANDA, Amphastar made a voluntary decision to propose a patented method as a quality-control procedure integral to its commercial manufacturing process. If Amphastar's maintenance of records documenting its use of that patented method as part of its commercial manufacturing procedures were enough to fall within Section 271(e)(1), that would permit drug manufacturers to infringe any number of patented inventions without consequences: the same regulations that require Amphastar to maintain records of

its manufacturing procedures require all drug manufacturers to maintain records of essentially all commercial manufacturing steps and activities.

Nothing in the statutory text of Section 271(e)(1) supports that result. And given the sheer scope of the manufacturing details the FDA requires be maintained by all manufacturers, adopting that approach would expand the safe harbor into an ocean. This Court should enforce the text as written and, with the benefit of a more complete record, hold that Section 271(e)(1) offers no protection for the commercial conduct here.

Finally, even if the safe harbor somehow protects Amphastar's *use* of Momenta's invention during commercial manufacture, that use is not Amphastar's only infringing act. Amphastar separately infringes each time it *offers to sell* and *sells* its enoxaparin that was made using Momenta's patented process. Those sales activities, which were not considered in *Momenta I*, fall outside the plain terms of the safe harbor: those sales activities are not "solely for uses reasonably related to the development and submission of information under" the FDCA. Indeed, records, if any, of those sales efforts are neither submitted nor maintained under the FDCA. And Amphastar's sales activities infringe under the plain terms of Section 271(g), which makes it an act of infringement to "offer[] to sell" or "sell[] . . . within the United States a product which is made by a process patented in the United States." 35 U.S.C. § 271(g). The district court concluded otherwise only

by adding a limitation found nowhere in the text, requiring that the product be made by a process that is practiced abroad. That legal error requires reversal.

Summary judgment of non-infringement should be reversed and the case remanded for further proceedings.

STATEMENT OF THE CASE

A. Factual Background

1. Momenta's patented method

Momenta manufactures and sells generic enoxaparin, an anticoagulant sold under the branded name Lovenox®. A12451-A12455. In July 2010, Momenta obtained FDA approval for its enoxaparin. A12452.

Enoxaparin is manufactured by starting with heparin, a naturally occurring anticoagulant made up of long chains of sugar molecules. A12343-A12431 at A12346. The process involves cleaving heparin into shorter chains called oligosaccharides. A12346. The particular cleaving method used to manufacture enoxaparin chemically modifies some of the individual sugars on the ends of the resulting shorter sugar chains. A12347. These non-naturally occurring sugars include a 1,6-anhydro ring structure, which is present at a particular end—the reducing end—of approximately 20% of enoxaparin's sugar chains. A12347-A12349; A12602-A12603.

Momenta developed a multi-step method of analyzing the interim drug substance during the manufacture of commercial enoxaparin to determine the presence of a structural feature unique to enoxaparin. A12451-A12452. Momenta's method separates the product of the early manufacturing steps that can be further formulated into final drug product from product that does not meet quality standards and may not proceed. A12432-A12450 at A12436-A12440.

Momenta's manufacturing method is claimed in U.S. Patent No. 7,575,886 ("'886 patent"). Claim 53 is representative. It has four steps: (1) exhaustively digesting an enoxaparin preparation (i.e., breaking down enoxaparin oligosaccharides into very short sub-chains), (2) performing a separation method to separate the sub-chains and determine the presence of the non-naturally occurring sugar that is a structural signature of enoxaparin, (3) comparing that determination to a reference standard, and (4) selecting a batch of intermediate product for further processing based on that comparison. A105(col.70:10-29); *see also* A102(col.64:40-57) (claim 6).

An enoxaparin manufacturer could use Momenta's patented method in a variety of ways. A manufacturer wishing to obtain FDA approval for its generic enoxaparin could use Momenta's method during the approval process to demonstrate that the result of its proposed manufacturing process is the same drug product as Lovenox®. To obtain FDA approval, a manufacturer must establish in

its ANDA that the active ingredient of the proposed generic drug product is the same as that of the branded drug. 21 U.S.C. § 355(j)(2)(A)(ii)(I); 21 C.F.R. § 314.94(a)(5)(i); A12581-A12582. The safe harbor exempts from infringement that use of a patented method. 35 U.S.C. § 271(e)(1).

Another potential use of Momenta's method—the use at issue here—is for commercial manufacturing purposes, as a quality-control procedure to ensure consistency in manufacturing. A12436-A12440. After FDA approval, manufacturers must perform a quality-control procedure on every batch of commercial drug substance to ensure that it conforms to established specifications for the drug before allowing it to proceed in the manufacturing process. 21 C.F.R. § 211.165. The FDA does not require manufacturers to use any particular quality-control procedure in their manufacturing process. Rather, ANDA applicants choose which manufacturing processes, including quality-control procedures, that they propose to use in commercial manufacturing, and the FDA can approve or reject the proposed procedures. A12255. NDA and ANDA applicants thus may propose to use either a non-patented or a patented quality-control procedure. A12255. After approval, the holders of an NDA or ANDA can change their manufacturing procedures with or without advance notice to the FDA, depending on the change. 21 C.F.R. § 314.70(a).

The record developed on summary judgment establishes that the FDA does not require the use of Momenta's patented manufacturing method to make and sell enoxaparin. Indeed, Momenta itself does not use the methods claimed in the '886 patent during manufacturing. A12356-A12362; A12452-A12454. Instead, Momenta proposed to the FDA use of a two-dimensional nuclear magnetic resonance spectroscopy ("2D-NMR") procedure during manufacturing, and the FDA approved that procedure. A12452-A12454. The 2D-NMR procedure does not involve a number of steps of Momenta's patented method. A12356-A12362; A12453-A12454.

2. The USP Monograph and optional <207> procedure

A written standard specifically describing enoxaparin is recorded in the United States Pharmacopeia ("USP") Enoxaparin Sodium Monograph. A12352-A12353; A12653-A12655. The USP Enoxaparin Sodium Monograph requires that 15-25% of the oligosaccharides have a 1,6-anhydro ring structure. A12653.

In 2009, long after Momenta developed its process and submitted its 2003 patent application (A54), the USP published a procedure that can be used to establish that a batch of enoxaparin meets the 15-25% requirement of the USP Monograph. A12657-A12663; A12665. This procedure is the General Chapter <207> Test for 1,6-Anhydro Derivative for Enoxaparin Sodium ("<207> procedure"). A12353; A12657-A12663. The <207> procedure involves

exhaustive digestion of a sample of enoxaparin in a particular way and for a particular length of time, followed by the use of a separation method to determine the percentage of sugar chains containing a 1,6-anhydro ring structure at the reducing end. A12353-A12354; A12657-A12663.

The standard of the USP Monograph for enoxaparin is distinct from the optional <207> procedure. While the FDA mandates that enoxaparin manufacturers must ensure that their drug product meets the standard of the USP Monograph, it does not require the use of any particular procedure for doing so. A12255. Thus, manufacturers are required to perform *a* procedure to determine whether a batch of enoxaparin meets the 15-25% requirement of the USP Monograph, but manufacturers are not required to use the particular <207> procedure to determine whether the 15-25% requirement is met. A12255.

Indeed, a USP bulletin observes that manufacturers "are always permitted to use alternative tests in accordance with Section 6.30 of USP General Notices." A12665. And the FDA's Compliance Policy Guide provides that where a manufacturer is making a drug listed in the USP, "neither the USP[] nor the [FDA's Current Good Manufacturing Practice] regulations necessarily require a firm to utilize, as a batch release test, the methods and procedures stated in the official compendia." A12680. What is required is that manufacturers use "suitable means, including adequate manufacturing process validation and control," to

ensure that "drug products conform to the appropriate compendial standards." A12680.

3. Amphastar's use of Momenta's methods to manufacture enoxaparin commercially and its sales of enoxaparin made by Momenta's method

Amphastar submitted an ANDA for its generic enoxaparin and received FDA approval on September 19, 2011. A12490-A12491. To prepare its ANDA to submit to the FDA, Amphastar used Momenta's process to establish that its drug is the "same" as Lovenox® and conforms to the USP Monograph for enoxaparin. Momenta has never accused of infringement Amphastar's use of Momenta's process to develop and prepare its ANDA for submission to the FDA.

In its ANDA, Amphastar chose to propose, as part of its commercial manufacturing process, that it would use a quality-control procedure that practices Momenta's methods to ensure that each commercial batch of its enoxaparin meets the 15-25% 1,6-anhydro requirement. The FDA approved Amphastar's ANDA with that proposed procedure. A12362-A12379. What Momenta accuses of infringement is Amphastar's subsequent use of Momenta's process in the course of

ordinary commercial manufacturing, and Amphastar's sales activities relating to the product made by that process.[1]

It is undisputed on the present record that Amphastar's approved 15-25% 1,6-anhydro procedure performs all the steps of at least claims 6 and 53 of the '886 patent. A12362-A12376. [redacted] Amphastar used its approved procedure as a step in its manufacturing process, to select batches of interim enoxaparin to be processed into finished drug product. A12365; A12437-A12440.

Approximately two months after receiving FDA approval, Amphastar revised this quality-control procedure, [redacted] A12398-A12403. It is undisputed on the present record that Amphastar's revised 15-25% 1,6-anhydro procedure performs all the steps of at least claims 6 and 53 of the '886 patent. A12403-A12409. [redacted]

[1] Because Amphastar's sales activities involve offers to sell and sales of a product made by a patented process rather than of a patented product itself, Momenta accuses those sales activities of infringement under Section 271(g) rather than Section 271(a).

Amphastar documents its use of these procedures during commercial manufacturing and maintains that documentation in its files. *E.g.*, A14125-A14438. This process of documentation and maintenance applies to *all* drug manufacturers, not just to this particular quality-control procedure, to enoxaparin, or to generic drugs. The FDCA and the FDA's Good Manufacturing Practice regulations provide that a manufacturer must document and maintain records of the use of substantially every step in the commercial manufacture of every batch of drugs that is produced for commercial sale. *See, e.g.*, 21 U.S.C. § 355(e), (i), (k); 21 C.F.R. §§ 211.180-211.194. For instance, for each batch of drugs, the manufacturer must create "[b]atch production and control records," which include detailed "[d]ocumentation that each significant step in the manufacture, processing, packing, or holding of the batch was accomplished," 21 C.F.R. § 211.188(b), and "complete data derived from all tests necessary to assure compliance with established specifications and standards," *id.* § 211.194(a).

Amphastar does not develop and submit to the FDA the records of any part of its commercial manufacturing process, including its quality-control procedures. Rather, like every other drug manufacturers' batch records, these records simply are "retained for at least 1 year after the expiration date of the batch." *Id.* § 211.180(a). The records must be "readily available for authorized inspection

during the retention period at the establishment where the activities described in such records occurred." *Id.* § 211.180(c).

Since approval by the FDA, Amphastar has offered for sale and sold [redacted] enoxaparin that was made using Momenta's patented methods. A13050-A13073; A13288-A13300. Sales and offers to sale have been made to wholesalers, group purchasing organizations, hospitals, and pharmacies. A13055-A13073. This sales activity occurred in direct competition with Momenta. Amphastar has no license to use Momenta's patented methods, yet it thus far has been permitted freely to reap the benefits of Momenta's research and inventive effort to compete directly with Momenta during the term of Momenta's patent.

B. Prior Proceedings

1. Preliminary injunction, appeal, and certiorari petition

a. In September 2011, two days after Amphastar received FDA approval for its enoxaparin, Momenta sued Amphastar, alleging infringement of the '886 patent. A12477-A12487. Nine days later, Momenta moved for a preliminary injunction. A282-A284. Relying on *Classen Immunotherapies, Inc. v. Biogen Idec*, 659 F.3d 1057 (Fed. Cir. 2011), the district court granted the preliminary injunction, concluding that Section 271(e)(1) does not apply because Amphastar's "alleged infringing activity involves use of plaintiffs' patented quality control

testing methods on each commercial batch of enoxaparin that will be sold after FDA approval." A1337-A1366 at A1359.

b. This Court expedited Amphastar's appeal of the preliminary injunction. Amphastar's primary argument for fitting within the text of Section 271(e)(1) was that the FDA purportedly mandated use of the <207> procedure, which practices Momenta's patented method. Brief for Amphastar at 44, *Momenta I* (Fed. Cir. Dec. 5, 2011). Momenta responded that *Classen* controlled and that, under that precedent, Amphastar's post-approval conduct was unprotected. Brief for Momenta at 38-40, *Momenta I* (Fed. Cir. Dec. 13, 2011). Neither party's brief addressed some of the central issues here—whether merely maintaining batch records of infringing use during commercial manufacturing constitutes "development and submission of information under" the FDCA, whether Amphastar's use is "solely" related to any such "submission," and whether Amphastar is liable for its separately infringing sales activity.

A divided panel of this Court vacated the preliminary injunction. *Momenta I*, 686 F.3d at 1361. The majority concluded Momenta was not "likely to succeed." *Id.* at 1352. Analyzing the text of Section 271(e)(1), the Court rejected the "pre-/post-approval distinction used by the district court," concluding that "the plain language of the statute is not restricted to pre-approval activities." *Id.* at 1358-59.

Because the limited preliminary-injunction record did not contain evidence rebutting Amphastar's assertion that the FDA mandates use of the <207> procedure, the panel majority accepted it as true: "Amphastar is required by the FDA to use" the <207> procedure "in order to ensure its enoxaparin is not adulterated." *Id.* at 1361. The Court went on to suggest that even if "non-infringing alternatives" were available, it would not matter because the safe harbor "does not mandate the use of a non-infringing alternative when one exists." *Id.* at 1359.

The majority pointed to regulations requiring Amphastar to maintain records of the quality-control procedures used to manufacture each batch of drug product. *Id.* at 1358 (citing 21 C.F.R. §§ 211.186, 211.188, 211.194). The majority reasoned that "the requirement to maintain records for FDA inspection satisfies the [safe harbor's] requirement that the uses be reasonably related to the development and submission of information to the FDA." *Id.* at 1357.

In dissent, then-Chief Judge Rader explained that "allow[ing] *continuous, commercial* infringing sales during any portion of the life of the patent" could not be squared with Section 271(e)(1). *Id.* at 1366. He observed that the majority opinion "rewrites the law to allow Amphastar to infringe Momenta's patent throughout *the entire life of Momenta's patent* and for the purpose of obtaining

profits on *commercial sales* of a product that *competes with the patentee*." *Id.* (emphasis in original).

c. Momenta petitioned for rehearing en banc, contending that the decision conflicted with *Classen*. Petition for Rehearing at 6-8, *Momenta I* (Fed. Cir. Sept. 4, 2012). Amphastar opposed, arguing that this case "involves the unusual situation where both Congress and the FDA have *mandated* the use of a particular test, specified in the official USP compendium." Response to Petition for Rehearing at 1, *Momenta I* (Fed. Cir. Oct. 15, 2012) (emphasis by Amphastar). Amphastar contended that the case's interlocutory nature poorly postured it for en banc review because the only issue was "whether there is a 'substantial question' about the merits of Momenta's infringement claim." *Id.* at 14. This Court denied rehearing.

d. Momenta sought Supreme Court review. Petition for a Writ of Certiorari, *Momenta Pharm., Inc. v. Amphastar Pharm., Inc.*, 133 S. Ct. 2854 (Feb. 15, 2013) (No. 12-1033). Momenta contended that *Classen* and *Momenta I* were irreconcilable, and yet neither decision correctly applied the statutory text. Momenta agreed with *Momenta I* that the text of Section 271(e)(1) did not support *Classen*'s bright-line pre-approval rule, but contended that *Momenta I* departed from the statutory text to the extent it treated records that were not submitted (or

ever intended to be submitted) under the FDCA as a Section 271(e)(1) "submission."

Amphastar responded that review of the interlocutory ruling was not warranted because Momenta had relied only on *Classen*'s "pre- and post-approval and pre- and post-marketing lines" and this Court thus "was never afforded the opportunity to review [Momenta's] newly reconfigured reading of the safe harbor provision." Brief in Opposition at 19-20, *Momenta Pharm., Inc. v. Amphastar Pharm., Inc.*, 133 S. Ct. 2854 (May 24, 2013) (No. 12-1033). As it had in this Court, Amphastar asserted that "Congress and the FDA have together commanded" use of the <207> procedure. *Id.* at 13.

Meanwhile, GlaxoSmithKline filed a certiorari petition in *Classen*, and the Supreme Court invited the Solicitor General to file a brief expressing the views of the United States. *GlaxoSmithKline v. Classen Immunotherapies, Inc.*, 133 S. Ct. 50 (2012). The Solicitor General contended that *Classen*'s bright-line rule did not correctly interpret Section 271(e)(1), as "[n]othing in the language of the statute links the availability of Section 271(e)(1)'s safe harbor to the timing of FDA marketing approval." Brief for United States as Amicus Curiae at 11, *GlaxoSmithKline* (Dec. 13, 2012) (No. 11-1078). But the Solicitor General did not endorse *Momenta I*'s suggestion that "information may be deemed 'submitted' to FDA if it is preserved in records that FDA regulations require a drug manufacturer

to make available for inspection by FDA on request"; he explicitly expressed no opinion on that issue. *Id.* at 20 n.4. The Solicitor General explained, however, that "the ordinary commercial exploitation of a patented invention is not 'reasonably related to the development and submission of information' for the FDA, even if such exploitation sometimes generates information useful to the FDA." *Id.* at 18. For example, "[a] drug maker's use of a patented invention in routine commercial activity is not immune from infringement liability merely because, for example, the company may periodically report adverse reactions to the FDA." *Id.*

The Supreme Court denied certiorari in both cases.

2. Summary judgment of non-infringement

a. In district court, Amphastar moved for summary judgment of non-infringement. A6749-A6773, A15220-A15230. Momenta opposed, offering additional evidence and legal support not in the preliminary-injunction record. A12273-A12297.

Momenta contended that the FDCA expressly distinguishes between information that is generated to be *submitted* to the FDA and records of ongoing, ordinary commercial manufacturing activity that must be *maintained* by the manufacturer. By its terms, Section 271(e)(1) exempts from infringement only uses that are solely and reasonably related to the development of information that the FDCA requires be submitted, not to records that merely must be maintained.

Momenta also proffered a detailed declaration from William Vodra, the primary author of the most recent revisions to the FDA's Good Manufacturing Practice regulations. A12251-A12272 at A12252. Mr. Vodra explained that "the [Good Manufacturing Practice] regulations require documentation of every aspect and step of the approved manufacturing process." A12264. Such documentation is required so that, in the event a drug product on the market is found not to comply with its specifications, the manufacturer can examine the complete process that was used on every batch of that drug product, to determine where and how the problem occurred. A12262. He also explained that neither the FDCA nor the FDA's Good Manufacturing Practice regulations authorize the FDA to order a manufacturer to submit these records to the FDA; rather, the FDA must come inspect the records at the site where they are maintained. A12263; 21 C.F.R. § 211.180(c).

Because of the sheer scope of the manufacturing details required to be documented and maintained, construing Section 271(e)(1) as protecting any commercial manufacturing activity subject to those requirements would permit manufacturers to "infringe any number of patented inventions without consequences." A12264. A manufacturer could, during ordinary commercial manufacturing, infringe any and all patented manufacturing methods, equipment

designs, and packaging processes—merely because it must document its use of such inventions in its Good Manufacturing Practice records. A12264-A12265.

Momenta also demonstrated that, contrary to Amphastar's assertions in this Court and the Supreme Court during the preliminary-injunction appeal, FDA regulations do not require Amphastar to use Momenta's specific patented process. A12255-A12256. In fact, as the undisputed record now establishes, Momenta was approved for commercial sale using a procedure that is markedly different from both the '886 claimed methods and the <207> procedure. A12356-A12362; A12453-A12454. [redacted]

Momenta opposed summary judgment on the additional ground that, even if Amphastar's *use* of Momenta's patented method were protected by the safe harbor, Amphastar's *sales of* and *offers to sell* enoxaparin were not. Those sales activities separately infringe under Section 271(g), which makes it an act of infringement to "offer[] to sell" or "sell[] . . . within the United States a product which is made by a process patented in the United States." 35 U.S.C. § 271(g). While the safe harbor could protect conduct that would otherwise infringe under Section 271(g) if the conduct fell within the terms of Section 271(e)(1), Amphastar's sales efforts do

not. Amphastar's liability under Section 271(g) for its sales activities was not considered in the preliminary-injunction appeal, and this Court has held that each infringing act must be separately considered for coverage by the safe harbor.

b. The district court, without engaging with Momenta's new evidence and legal support, held that Section 271(e)(1) exempts from infringement Amphastar's commercial use of Momenta's patented methods. A7-A9; *Momenta Pharm., Inc. v. Amphastar Pharm., Inc.*, 962 F. Supp. 2d 348 (D. Mass. 2013). The district court reasoned that this Court's preliminary-injunction opinion "expressly held that the maintenance of records for FDA inspection 'satisfies the requirement that the uses be reasonably related to the development and *submission* of information to the FDA.'" A8-A9 (emphasis added by district court) (quoting *Momenta I*, 686 F.3d at 1357).

The district court also rejected Momenta's argument that Amphastar's conduct is not "solely" related to the development and submission of information. A9. Relying on a footnote in the preliminary-injunction opinion, the district court stated: "Unfortunately for plaintiffs, the Federal Circuit found that such an argument is 'not a tenable reading of the statute' and is 'contrary to precedent.'" A9 (quoting *Momenta I*, 686 F.3d at 1360 n.2).

The district court further held that Amphastar's sales activity does not infringe under Section 271(g). A9-A11. Relying on a statement from a dissenting

opinion, the district court concluded that Section 271(g) "'requires importation or sale of the product of a patented process *practiced abroad*, before infringement can be established under that provision.'" A10 (emphasis added) (quoting *Cardiac Pacemakers, Inc. v. St. Jude Med., Inc.*, 576 F.3d 1348, 1369 (Fed. Cir. 2009) (en banc) (Newman, J., dissenting)). "Because there is no suggestion that Amphastar manufactures enoxaparin abroad, § 271(g) is inapplicable in this case." A10-A11.

3. Denial of motion to amend infringement contentions

Before the district court's summary-judgment decision, Momenta moved for leave to amend its infringement contentions to add two additional Amphastar procedures about which Momenta learned in discovery: Amphastar's Disaccharide Building Block (DBB) Procedure and its batch-to-batch comparisons. A14439-A14441. Both of these procedures infringe Momenta's '886 patent.

The DBB Procedure follows the same initial steps as the 1,6-Anhydro 15-25% Procedure, [redacted]

[redacted]

Performing the DBB Procedure practices each step of at least claims 6 and 53 of the ’886 patent. A12386-A12394.

The batch-to-batch comparisons are performed in addition to the procedures that Amphastar performs on each batch of enoxaparin. This procedure involves comparing the results of Amphastar’s quality-control procedure across multiple batches. It is used to determine whether there are any untoward trends and ensure that Amphastar’s commercial manufacturing process remains in control, that it produces a consistent product, and that any necessary adjustments are made. A12395, A12409-A12410; A13133-A13134; A13716, A13726-A13741, A13748-A13749. When making batch-to-batch comparisons, Amphastar performs each step of claim 1 of the ’886 patent. A12394-A12395, A12409-A12410. Although the first three steps of claim 1 are the same as those of claims 6 and 53, the last step differs and requires “determining the presence of the structural signature associated with the non naturally occurring sugar associated with peak 9 of FIG. 1 in a second batch of enoxaparin.” A102(col.64:3-5). There is no evidence that Amphastar proposed this procedure to the FDA or that the FDA approved it.

Amphastar has produced no document that suggests that it maintains records of its batch-to-batch comparisons.

Momenta could not have accused these additional procedures earlier because Amphastar wrongfully withheld evidence of its DBB Procedure in discovery and produced no documentation of its batch-to-batch comparisons. Indeed, Amphastar's withholding of the existence of the DBB Procedure was part of a pattern of discovery misconduct that culminated in Amphastar being sanctioned. The district court found that Amphastar "disobeyed" court orders compelling production of, *inter alia*, documents concerning Amphastar's quality-control procedures and sanctioned Amphastar. A24-A26; *see* A28, A30-A31; A33-A42.

Nevertheless, the district court denied Momenta's motion to amend its infringement contentions. A11-A14. The court stated that it was "concerned by Momenta's allegations that the documents necessary to discover the additional tests were intentionally concealed by defendants, possibly in violation of a court order." A12. But the district court concluded that "the proposed amendments would be futile in any event." A12. The district court reasoned that the Section 271(e)(1) safe harbor protects Amphastar's use of the DBB test because Amphastar maintains records of its use of the procedure. A12-A13. With respect to batch-to-batch comparisons, the district court stated that it was "skeptical that the so called batch-to-batch test is even a separate testing procedure." A13. Thus, rather than

determine whether use of that procedure performs each step of claim 1 and evaluate separately whether that activity is protected by the safe harbor, the district court simply held that "a mere comparison of already produced data could not possibly infringe the '866 patent." A14.

4. This Court's denial of summary affirmance

Momenta appealed. Amphastar moved for summary affirmance, arguing that *Momenta I* already determined that Amphastar's release tests are protected by the Section 271(e)(1) safe harbor. Dkt. 32-1 at 2, 8. Momenta responded that the preliminary-injunction decision is not binding in this final-judgment appeal, which involves record evidence, legal support, and additional conduct not before the Court in *Momenta I*. Dkt. 41 at 8-9.

This Court denied Amphastar's motion for summary affirmance. Dkt. 43.

SUMMARY OF ARGUMENT

I. The Section 271(e)(1) safe harbor does not protect Amphastar's use of Momenta's patented method. To the extent *Momenta I* suggested otherwise, that preliminary-injunction decision is not binding. Indeed, the record now includes further development of legal arguments and establishes that assertions made by Amphastar and accepted by the Court in the preliminary-injunction appeal—including Amphastar's assertion that the FDA required it to use an infringing method—were not correct.

The present record demonstrates that Amphastar's use is not exempt. The safe harbor's scope is governed by the text of Section 271(e)(1), which exempts only activities that are "solely for uses reasonably related to the development and submission of information" under the FDCA. The FDCA and its implementing regulations expressly distinguish between "submitting" information to the FDA and "maintaining" records. Here, the fact that the FDCA requires Amphastar merely to "maintain," not to "submit," records documenting its quality-control procedures precludes Amphastar's reliance on the safe harbor.

That Section 271(e)(1) protects conduct "reasonably related" to the development and submission of information does not help Amphastar, as Amphastar's use is related only to records that must be maintained, not information developed for any submission. In any event, Amphastar's commercial use of Momenta's method is not "solely" related to the development and submission of information under the FDCA.

If the safe harbor applied here simply because Amphastar maintains commercial-manufacturing records, that would expand the safe harbor to exempt from infringement the commercial use of virtually any patented method or composition in the course of producing a drug product. That cannot be what Congress intended.

II. Even were Amphastar's *use* of Momenta's method protected by the safe harbor, summary judgment still should be reversed because Amphastar's sales activities separately infringe under Section 271(g). Those sales activities fall outside the plain terms of Section 271(e)(1); indeed, records, if any, of those sales efforts are neither submitted nor maintained under the FDCA. Section 271(g) makes it an act of infringement to "offer[] to sell" or "sell[] . . . within the United States a product which is made by a process patented in the United States." Amphastar's conduct falls squarely within the statutory text: Amphastar uses Momenta's patented method to manufacture its enoxaparin, and then it offers to sell and sells that enoxaparin in the United States. The district court held that Section 271(g) requires that the patented process have been practiced abroad, but neither the statutory text nor this Court's precedent contains that requirement.

III. Denial of Momenta's motion for leave to amend its infringement contentions also should be reversed because the district court legally erred in holding that amendment would be futile. Neither the DBB Procedure nor the batch-to-batch comparisons is required by the FDA.

STANDARD OF REVIEW

This Court reviews summary judgment decisions under regional circuit law. *MicroStrategy Inc. v. Business Objects, S.A.*, 429 F.3d 1344, 1349 (Fed. Cir. 2005).

The First Circuit reviews such decisions de novo. *Johnson v. University of Puerto Rico*, 714 F.3d 48, 52 (1st Cir. 2013).

Issues of statutory construction are reviewed without deference. *Doyon, Ltd. v. United States*, 214 F.3d 1309, 1314 (Fed. Cir. 2000).

Decisions whether to allow an amendment to pleadings after the scheduling order deadline are reviewed under regional circuit law. *Aventis Pharma S.A. v. Hospira, Inc.*, 675 F.3d 1324, 1333 (Fed. Cir. 2012). The First Circuit reviews the denial of such motions for abuse of discretion. *O'Connell v. Hyatt Hotels of Puerto Rico*, 357 F.3d 152, 154-55 (1st Cir. 2004). "Where a legal error is committed, there is by definition an abuse of discretion." *Top Entm't, Inc. v. Torrejon*, 351 F.3d 531, 533 (1st Cir. 2003).

ARGUMENT

I. AMPHASTAR'S USE OF MOMENTA'S PATENTED METHOD FALLS OUTSIDE SECTION 271(e)(1)'S SAFE HARBOR

The district court erred in holding, on the more complete factual and legal record now developed, that the safe harbor provides immunity to Amphastar for its commercial use of Momenta's patented method. By its plain terms, Section 271(e)(1) does not apply here.

A. The Preliminary-Injunction Decision Is Not Binding Here

As an initial matter, this Court is not bound by its decision at the preliminary-injunction stage of this case.

1. An appellate court's preliminary-injunction opinion is not binding in later proceedings

This Court has emphasized that "[a]n appellate court's preliminary injunction opinion has no conclusive bearing at the trial on the merits and is not binding on a subsequent panel." *Glaxo*, 376 F.3d at 1346. Preliminary-injunction proceedings are "only the first round." *Illinois Tool Works, Inc. v. Grip-Pak, Inc.*, 906 F.2d 679, 681 (Fed. Cir. 1990). Because of the abridged nature of preliminary-injunction proceedings, "all findings of fact and conclusions of law at the preliminary injunction stage are subject to change." *Purdue Pharma L.P. v. Boehringer Ingelheim GmbH*, 237 F.3d 1359, 1363 (Fed. Cir. 2001); *Ugine & ALZ Belgium v. United States*, 452 F.3d 1289, 1294 (Fed. Cir. 2006) (preliminary-injunction holdings are "necessarily tentative"). Indeed, not only do preliminary-injunction decisions by an appellate court not bind a subsequent panel, they do not bind the district court in the same action. *Glaxo*, 376 F.3d at 1346.

This established principle flows from the nature of preliminary-injunction proceedings. At the preliminary-injunction stage, courts consider only likelihood of success. *University of Tex. v. Camenisch*, 451 U.S. 390, 395 (1981). "The purpose of a preliminary injunction is merely to preserve the relative positions of the parties until a trial on the merits can be held." *Id*. "Given this limited purpose, and given the haste that is often necessary if those positions are to be preserved, a preliminary injunction is customarily granted on the basis of procedures that are

less formal and evidence that is less complete than in a trial on the merits." *Id.* Because of the "significant procedural differences" between preliminary-injunction proceedings and the trial on the merits, parties may introduce new evidence and legal support at the merits stage. *Id.* at 394.

This rule applies even for purely legal questions. *Id.* at 395 ("conclusions of law made by a court" in deciding a preliminary injunction motion are not later binding). For example, in *Glaxo*, this Court construed a claim term afresh in the appeal from the final judgment, even though the Court previously had construed the same term in the preliminary-injunction appeal. *Glaxo*, 376 F.3d at 1346; *Glaxo Grp. Ltd. v. Apotex, Inc.*, 64 F. App'x 751, 754 (Fed. Cir. 2003).

2. Momenta I *is not binding here*

These principles apply fully here. The preliminary-injunction proceedings were heard quickly, with minimal, expedited discovery, and on a truncated record. Appellate briefing likewise was expedited. Order, *Momenta I* (Fed. Cir. Nov. 17, 2011), Dkt. 5. On the merits, this Court reviewed only the question whether Momenta had "sufficiently established a reasonable likelihood of success" to warrant equitable relief. *Momenta I*, 686 F.3d at 1352.

The record has now been more fully developed. It establishes that certain factual and legal assertions made by Amphastar in the preliminary-injunction appeal were not correct. For example, undisputed evidence flatly contradicts

Amphastar's primary argument for application of the safe harbor: that the FDA purportedly mandated Amphastar's use of Momenta's specific patented method for commercial manufacturing of enoxaparin.

Amphastar argued that it was "required by the FDA to perform" the USP <207> procedure "on every single batch of generic enoxaparin it produces before any is sold or administered to patients." Brief for Amphastar, *Momenta I*, *supra*, at 44. Indeed, Amphastar argued that the safe harbor applied because the USP <207> procedure was "the only FDA-approved method" that would "satisfy the FDA-mandated testing," and "use of a different testing method would *not* meet the FDA's mandated proof." Reply Brief for Amphastar at 5, 7, *Momenta I* (Fed. Cir. Dec. 20, 2011).

On the limited record before it, this Court accepted Amphastar's assertion as true. *Momenta I*, 686 F.3d at 1361. The panel stated that "Amphastar is required to conduct a laboratory determination of identity and strength of the active ingredient for each batch of enoxaparin," and that "[t]his test must be done according to the patented methods described in an official compendium," i.e., the USP. *Id.* at 1358. The panel reasoned that because "Amphastar is required by the FDA to use this test in order to ensure its enoxaparin is not adulterated," "[t]his testing . . . therefore falls squarely within the scope of the safe harbor." *Id.* at 1361.

But undisputed evidence in the current record demonstrates that Amphastar was not required to use, and in fact did not use, the <207> procedure. The FDA requires the use of *a* procedure during commercial manufacturing to determine whether each batch of drug product meets the requirement in the USP Monograph that 15-25% of enoxaparin oligosaccharides include a 1,6-anhydro ring structure. But the FDA does not require the use of any particular quality-control method. A12255. Neither Momenta's patented method nor the USP <207> procedure (which is published separately from the USP Monograph) is required. A12255-A12256. In fact, Momenta's enoxaparin was approved for commercial sale using a procedure that is markedly different from both the '886 claimed methods and the <207> procedure. A12356-A12362; A12453-A12454. [redacted]

To be sure, *Momenta I* suggested that it did not matter whether the FDA required use of the particular method covered by Momenta's patent. 686 F.3d at 1359 ("The safe harbor . . . does not mandate the use of a non-infringing alternative when one exists."). But *Momenta I* also recognized that otherwise infringing conduct was protected only if it fell within the plain terms of Section 271(e)(1). *Id.* (noting that "[t]he only limitation in the safe harbor" is the words of

the statutory provision). The current record demonstrates that Amphastar's conduct does not.

For example, in the preliminary-injunction appeal, neither party's briefs addressed whether Amphastar's maintenance of batch records documenting its commercial manufacturing activities constitutes a "submission of information under" the FDCA. The district court had relied on *Classen* in granting the preliminary injunction. A1359. Amphastar argued that "the district court's categorical pre- and post-approval testing line lacks any anchor in statutory text." Brief for Amphastar, *Momenta I*, *supra*, at 44. Momenta responded that the district court correctly applied the rule announced in *Classen*, which was controlling precedent. Brief for Momenta, *Momenta I*, *supra*, at 38-40. This Court thus did not have the benefit of briefing on the statutory distinction between "submission" of information and "maintenance" of records when it "consider[ed] this information 'submitted' for purposes of the statute." *Momenta I*, 686 F.3d at 1357. Indeed, when Momenta sought Supreme Court review, Amphastar responded that Momenta's argument was a "[n]ew" one that this Court "was never afforded the opportunity to review." Brief in Opposition, *supra*, at 19-20.

If any precedent governs here, it is *Classen*. *Newell Cos. v. Kenney Mfg. Co.*, 864 F.2d 757, 765 (Fed. Cir. 1988) ("Where there is direct conflict [between two decisions of this Court], the precedential decision is the first."). Under

Classen's rule that Section 271(e)(1) "is limited to activities conducted to obtain pre-marketing approval," 659 F.3d at 1070, Amphastar's post-approval use is infringing.

But what should govern is the statutory text, under which Amphastar's use is not protected. This appeal affords the opportunity to elucidate the correct application of that text. This Court should clarify the scope of Section 271(e)(1) and limit the safe harbor to its proper bounds.

B. Section 271(e)(1) Does Not Protect Amphastar's Use Of Momenta's Method For Commercial Manufacturing

1. The statutory text governs

As this Court explained in *Momenta I*, the statutory text governs the scope of the safe harbor of Section 271(e)(1). *Momenta I*, 686 F.3d at 1353-54. "'[A]ll statutory construction cases . . . begin with the language of the statute.'" *Id.* at 1353 (alteration in original) (quoting *Barnhart v. Sigmon Coal Co.*, 534 U.S. 438, 450 (2002)). "[W]here, as here, the statute's language is plain, 'the sole function of the courts is to enforce it according to its terms.'" *United States v. Ron Pair Enters., Inc.*, 489 U.S. 235, 241 (1989) (quoting *Caminetti v. United States*, 242 U.S. 470, 485 (1917)). Indeed, the Supreme Court has twice so instructed with respect to the safe harbor in particular. *See Merck KGaA v. Integra Lifesciences I, Ltd.*, 545 U.S. 193, 206-07 (2005); *Eli Lilly*, 496 U.S. at 665-67, 679.

Section 271(e)(1) provides that "[i]t shall not be an act of infringement to . . . use . . . within the United States . . . a patented invention . . . solely for uses reasonably related to the development and submission of information under a Federal law which regulates the manufacture, use, or sale of drugs." 35 U.S.C. § 271(e)(1). As *Momenta I* explained, the text of Section 271(e)(1) does not draw a distinction between pre- and post-approval activities. 686 F.3d at 1355. Nor is it limited to activities involving an ANDA. *Id.* Rather, the phrase "'a Federal law which regulates the manufacture, use, or sale of drugs'" is "broad enough to encompass submissions made pursuant to the Federal Food, Drug, and Cosmetic Act." *Id.* (quoting 35 U.S.C. § 271(e)(1)).

As *Momenta I* also recognized, the language in Section 271(e)(1) must be interpreted in light of the "entire statutory scheme of regulation." *Eli Lilly*, 496 U.S. at 666; *see Momenta I*, 686 F.3d at 1354 (statutory interpretation focuses on "'the language itself, the specific context in which the language is used, and the broader context of the statute as a whole'" (quoting *Robinson v. Shell Oil Co.*, 519 U.S. 337, 341 (1997))). By its terms, the safe harbor is limited to activity related to "development and submission of information *under a Federal law*." 35 U.S.C. § 271(e)(1) (emphasis added). Congress thus directed that federal law governs what constitutes a "submission." The law at issue here is the FDCA.

2. The statutory scheme distinguishes between the "submission of information under" the FDCA and a requirement merely to "maintain" records

The text of the FDCA expressly distinguishes between two types of activity: "submitting" information to the FDA and "maintaining" records for possible FDA inspection. A12922-A13016. Specifically, the FDCA uses the terms "submit," "submitted," or "submission" in multiple provisions regulating new drug, generic drug, and other efforts to obtain regulatory approvals. Companies desiring to market a new drug or a generic version of an approved drug must "submit" an NDA or ANDA. A12253-A12257; *see* 21 U.S.C. § 355(b)(1) ("Such person shall *submit* to the Secretary as a part of the application . . . ."), (b)(2) ("An application *submitted* . . . ."), (b)(3)(D)(i) ("an application that contains bioavailability or bioequivalence studies has been *submitted*"), (i)(1)(A) ("the *submission* to the Secretary, before any clinical testing of a new drug is undertaken, of reports . . . of preclinical tests . . . of such drug adequate to justify the proposed clinical testing"), (j)(2)(B)(iv)(I) (notice must "state than an [ANDA] application . . . has been *submitted*"), (j)(2)(C) ("If a person wants to *submit* an abbreviated application for a new drug which has a different active ingredient or whose route of administration, dosage form, or strength differ from that of a listed drug, such person shall *submit* a petition to the Secretary . . . ."), (j)(4)(F), (G), (H) ("information *submitted* in the application") (emphases added).

The FDCA also contemplates that drug manufacturers may submit certain information to the FDA *after* approval. But these provisions also involve information developed and submitted for agency approval or study, not simply the maintenance of commercial manufacturing records for possible agency inspection. For example, a manufacturer may "submit to the Secretary" a proposed change to manufacturing methods or a new use of the drug. 21 U.S.C. § 355(b)(1); *see id.* § 355(a) (requiring approval for any new drug); *id.* § 321(p) (defining "new drug" as including new uses); *see also* 21 C.F.R. § 314.70 (prescribing circumstances requiring "submission" of a "supplement" to an approved application); *id.* § 314.71 (specifying procedures to "submit a supplement"); *id.* § 314.97 (providing requirements for "submission of supplemental applications" with respect to approved ANDAs); A12258.

The FDA also may require the manufacturer to conduct post-approval studies and to submit the results to the FDA. For example, the FDA may designate certain new drugs for "fast track" review and accelerated approval—which may grant FDA approval contingent on the applicant "conduct[ing] appropriate postapproval studies." 21 U.S.C. § 356(c)(2)(A). And the FDA may require a manufacturer to "conduct a postapproval study . . . or a postapproval clinical trial" to investigate unexpected safety-related issues with its product and to "submit a timetable for completion of the study or clinical trial." *Id.* § 355(o)(3)(A), (E)(ii).

The FDA also may require changes to a drug's labeling, in which case the manufacturer must "submit a supplement proposing changes to the approved labeling to reflect the new safety information." *Id.* § 355(o)(4)(B)(i). These types of activities involve development of information for submission, not merely documenting use of regular processes in records to be maintained by the manufacturer.

The plain meaning of "submission" as used in this context is the act of presenting something to another for approval or study. Oxford English Dictionary (3d ed. 2012) (defining "submission" as "[t]he action or an act of submitting something to another (freq. a higher authority) for decision or consideration"[2] and "submit" as "to present (something) to a person for criticism, consideration, approval, action, etc."[3]); Webster's New International Dictionary of the English Language 2227 (3d ed. 1993) (defining "submission" as "an act of submitting something (as for consideration, inspection, or comment)" and "submit" as "to send or commit for consideration, study, or decision"); Webster's Ninth New Collegiate Dictionary 1175 (1990) (defining "submission" as "an act of submitting something (as for consideration or inspection)" and "submit" as "to present or propose to another for review, consideration, or decision").

[2] http://www.oed.com/view/Entry/192823?redirectedFrom=submission.

[3] http://www.oed.com/view/Entry/192831?redirectedFrom=submit.

In stark contrast, the FDCA uses the word "maintain" to describe the activity at issue here: a manufacturer's obligation to keep records related to the ordinary course of commercial activities, including records documenting all manufacturing steps (including quality-control procedures) of an already-approved drug. Manufacturers are required to "maintain" whatever records the FDA Good Manufacturing Practice regulations require. *See, e.g.*, 21 U.S.C. § 355(e)(5) ("The Secretary may also . . . withdraw the approval of an application submitted under subsection (b) or (j) of this section with respect to any drug under this section if the Secretary finds (1) that the applicant has failed to establish a system for *maintaining* required records, or has repeatedly or deliberately failed to *maintain* such records . . . ."), (k)(1) ("In the case of any drug for which an approval of an application filed under subsection (b) or (j) of this section is in effect, the applicant shall establish and *maintain* such records . . . as the Secretary may . . . prescribe . . . ."), (k)(2) ("Every person required under this section to *maintain* records . . . shall, upon request of an officer or employee designated by the Secretary, permit such officer or employee at all reasonable times to have access to and copy and verify such records.") (emphases added); *see also* 21 C.F.R. § 211.180.

The FDCA uses "maintain" consistent with its plain meaning, which is different from the meaning of "submit." "Maintain" means to keep in existence or

to keep within one's possession. Black's Law Dictionary (9th ed. 2009) ("[t]o continue in possession of (property, etc.)"; Oxford English Dictionary ("[t]o keep up, preserve, cause to continue in being"; "to guard from loss or deterioration"; "to preserve in existence")[4]; Webster's New International Dictionary 1362 ("preserve from failure or decline"); American Heritage Dictionary of the English Language 1084 (3d ed. 1992) ("[t]o keep in an existing state; preserve or retain"); Webster's Ninth New Collegiate Dictionary 718 ("to keep in an existing state").

Records that are required to be maintained are not submitted to the FDA. Rather, the FDA regulations provide that these batch records must be "retained" by the manufacturer, "at the establishment where the activities described in such records occurred." 21 C.F.R. § 211.180(a), (c). The primary purpose of requiring the creation and maintenance of these records is so that manufacturers retroactively can examine the complete process used to produce any single batch of drug product, not for any approval or study by the FDA. A12262.

Although the FDA may inspect the records at the location where they are maintained, 21 C.F.R. § 211.180(c), and the manufacturer is required to "permit access" to the records, 21 U.S.C. § 331(e), the FDCA does not authorize the FDA to order a company to submit the records required under the Good Manufacturing

[4] http://www.oed.com/view/Entry/112562?rskey=ddzVI1&result=2&isAdvanced=false.

Practice regulations. A12263. As the primary author of the latest complete revision to the FDA's Good Manufacturing Practice regulations explained, the FDA interprets the absence of such authorization in the FDCA to mean that the FDA lacks the authority to require submission of batch records. A12263.

Given this textual distinction in the FDCA between "submission" and "maintenance," the phrase "submission of information under" the FDCA in Section 271(e)(1) should be interpreted accordingly. By its terms, Section 271(e)(1) does not protect the mere maintenance of records of routine commercial activity. This does not mean that the safe harbor is limited to pre-approval activity. *Momenta I*, 686 F.3d at 1354-55. Nor does it mean the safe harbor's protection is limited "to the dire situation where the patented invention is the only way to develop and submit the information." *Id.* at 1359. But it does mean that the safe harbor is limited to activities that involve the "development and submission of information under" a federal law for regulatory approval or study, not to regular manufacturing activity of which records are maintained.

This textual distinction reflects the purpose animating the safe harbor. Activity reasonably related to submissions of NDAs, ANDAs, and other regulatory information for approval or study by the FDA is precisely what the safe harbor was intended to exempt. *Eli Lilly*, 496 U.S. at 671. The safe harbor was enacted to "allow[] competitors, prior to the expiration of a patent, to engage in otherwise

infringing activities necessary to obtain regulatory approval." *Id.*; *see Amgen, Inc. v. ITC*, 565 F.3d 846, 852 (Fed. Cir. 2009) ("congressional purpose of removing patent-based barriers to proceeding with federal regulatory approval of medical products"); *see also* H.R. Rep. No. 98-857, pt. 1, at 45 (1984). But the safe harbor is not a free pass to use the patented invention for ordinary commercial conduct: one must either wait until the patent expires or pay the patentee for the use of its invention.

3. The mere maintenance of commercial batch records is not "reasonably related" to any "submission"

It is true, as *Momenta I* explained, that Section 271(e)(1) does not require "that the use of the patented invention must necessarily result in submission of information to the FDA." 686 F.3d at 1356. The Hatch-Waxman safe harbor encompasses activities that are solely for uses "*reasonably related* to the development and submission of information" under a law such as the FDCA. 35 U.S.C. § 271(e)(1) (emphasis added). As this Court and the Supreme Court have explained, by using "reasonably related," Congress left "adequate space for experimentation and failure on the road to regulatory approval." *Merck*, 545 U.S. at 207. Thus, patented inventions may be used without liability in the course of developing information for a potential submission under the FDCA, even if the result of that use is not itself submitted, and indeed even if no submission ultimately is made. "As long as the accused infringer 'has a reasonable basis for

believing' that use of the patented invention might yield information that 'would be appropriate to include in a submission to the FDA, that use is "reasonably related" to the "development and submission of information under . . . Federal law."'" *Momenta I*, 686 F.3d at 1356-57 (quoting *Merck*, 545 U.S. at 207 (quoting 35 U.S.C. § 271(e)(1))).

But that leeway does not help Amphastar here. Amphastar's commercial use of Momenta's method yields information that is appropriate to include in records that the FDCA requires merely be *maintained* by Amphastar. The use of "reasonably related" does not extend protection to the mere maintenance of records of post-approval, commercial activity—with no intention of submitting them to the FDA. As the United States explained to the Supreme Court, "the ordinary commercial exploitation of a patented invention is not 'reasonably related to the development and submission of information' for the FDA, even if such exploitation sometimes generates information useful to the FDA." Brief for United States, *GlaxoSmithKline*, *supra*, at 18.

4. In any event, Amphastar's infringing conduct is not "solely" for protected activity

Even if Amphastar's maintenance of manufacturing records were deemed to be reasonably related to a submission to the FDA, Amphastar's commercial use of Momenta's method still would fall outside the plain language of Section 271(e)(1) because it is not "solely" related to the development and submission of information

to the FDA. 35 U.S.C. § 271(e)(1).[5] The plain meaning of "solely" is "[o]nly, merely, exclusively." Oxford English Dictionary. Thus, the word "solely" in the statute makes clear that even if a patented invention is used for a purpose reasonably related to the development and submission of information to the FDA, other uses of the invention are not protected under the safe harbor.

This view of the "solely" requirement is supported by decisions of this Court and the Supreme Court, which have explained that each use of a patented invention "'must be evaluated separately to determine whether the [Section 271(e)(1)] exemption applies.'" *Amgen*, 565 F.3d at 852 (quoting *Merck*, 545 U.S. at 200). "*Merck* set careful boundaries to the exemption, requiring separate review of all studies for which the exemption was claimed." *Id.* at 853. While "scientific studies" that may lead to an FDA submission are exempt from infringement, "it is apparent that commercial and marketing studies are more clearly subject to separate evaluation for application of the exemption." *Id.* at 852.

The history of Section 271(e)'s enactment supports this textual reading of "solely." As the Supreme Court has explained, Section 271(e)(1) was enacted in

[5] Although a footnote in the preliminary-injunction decision suggested that the "solely" limitation was met, that passing conclusion was based on Amphastar's now-disproven assertion that the FDA requires use of Momenta's method. *Momenta I*, 686 F.3d at 1360 n.2 (explaining that the parties did "not argue that FDA-mandated quality control testing during manufacturing is not done 'solely' for purposes of developing and submitting information to the FDA").

response to *Roche Products, Inc. v. Bolar Pharmaceutical Co.*, 733 F.2d 858 (Fed. Cir. 1984). *Eli Lilly*, 496 U.S. at 670. Section 271(e)(1) addresses the holding in *Roche* that "the experimental use of a drug product . . . constitutes patent infringement, even though the *only* purpose of the experiments is to seek FDA approval for the commercial sale of the drug." H.R. Rep. No. 98-857, pt. 1, at 45-46 (emphasis added). Congress enacted Section 271(e)(1) to overrule that holding and allow uses that are solely for protected activities, such as seeking regulatory approval. *Eli Lilly*, 496 U.S. at 671.

Indeed, as the United States has explained, if a manufacturer "makes multiple 'uses' of a patented invention (*e.g.*, by selling a patented drug commercially while simultaneously administering it to research subjects during a controlled study), one 'use[]' may provide a basis for infringement liability even though the other falls within the safe harbor." Brief for United States, *GlaxoSmithKline*, *supra*, at 17. Thus, "[i]f the FDA has approved a drug for acne, for example, and its manufacturer separately conducts a clinical trial of the same drug as a treatment for melanoma, the clinical trial . . . will be protected," "but not the routine sales of the drug for acne treatment." *Id.* at 18. Furthermore, "[a] drug maker's use of a patented invention in routine commercial activity is not immune

from infringement liability merely because, for example, the company may periodically report adverse reactions to the FDA." *Id.*[6]

Here, Amphastar's use is not "[o]nly, merely, exclusively" (if at all) for developing and submitting information to the FDA. Oxford English Dictionary. Amphastar uses Momenta's method for the commercial manufacture of a product for sale. That use thus falls outside the plain text of Section 271(e)(1).

5. The district court's holding, if adopted by this Court, would immunize a wide swath of infringing commercial conduct

Here, the district court noted the distinction between the ordinary meanings of "submission" and "maintenance." A8. Yet it brushed that distinction aside: "[t]hese definitions do not, however, negate the fact that the Federal Circuit expressly held that the maintenance of records for FDA inspection 'satisfies the requirement that the uses be reasonably related to the development and *submission* of information to the FDA.'" A8-A9 (emphasis added by district court) (quoting *Momenta I*, 686 F.3d at 1357). And the district court rejected Momenta's reliance on "solely" based on a footnote in the preliminary-injunction opinion. A9. That

[6] The district court's reliance (A9) on *AbTox, Inc. v. Exitron Corp.*, 122 F.3d 1019 (Fed. Cir. 1997), is misplaced. There, the Court held that if the accused "activity is reasonably related to obtaining FDA approval," Section 271(e)(1) exempts it regardless of the accused infringer's "underlying purposes" or "intent." *Id.* at 1030. *AbTox* did not suggest that ordinary, commercial, post-approval uses could be "solely" related to the development and submission of information under the FDCA.

was error. The district court should not have entered final judgment without considering the complete record and determining anew whether Amphastar's mere maintenance of records of regular commercial activity fell within Section 271(e)(1). *See supra* Part I.A. For all the reasons set forth above, it does not. The district court's contrary conclusion turns the meaning of "submission" on its head and reads the word "solely" out of the statute.

Moreover if, as the district court held, Amphastar's "maintenance of records for FDA inspection" (A8) were sufficient to exempt Amphastar's conduct from infringement, that would expand the safe harbor into an ocean. As explained below, the batch records that Amphastar is maintaining are no different from the records FDA regulations mandate be maintained for essentially *all* commercial manufacturing activities for all pharmaceutical products, both branded and generic. If Congress had intended such broad immunity from the general principles of patent infringement, it surely would have said so explicitly.

FDA regulations require companies to maintain a huge swath of records memorializing compliance with each step in an approved manufacturing process. 21 C.F.R. §§ 211.180 *et seq.* These batch records must include "[d]ocumentation that each significant step in the manufacture, processing, packing, or holding of the batch was accomplished," *id.* § 211.188(b), and "complete data derived from all tests necessary to assure compliance with established specifications and

standards," *id.* § 211.194(a). The records must describe with specificity, for each batch of drug product, each component used, the procedures used to make the batch (i.e., the master recipe for the drug product), the identity of each piece of equipment used to make, package, and label the product, and the laboratory procedures used for testing initial components, in-process materials, and finished products. A12261; 21 C.F.R. §§ 211.186, 211.188, 211.194. Such details must be recorded and the record maintained until one year after a batch's expiration date so that a manufacturer may examine retroactively the complete process used to produce any single batch of drug product and determine the source of any problem that occurred during manufacturing. A12262.

If the safe harbor were read to exempt Amphastar's use of Momenta's method simply because of Amphastar's maintenance of records documenting such use, no principled distinction would preclude the safe harbor also from encompassing the commercial use of *any* patented method to produce a drug product. The use of approved manufacturing processes—including formulation processes, weighing and compounding processes, sterilization and cleaning techniques, computerized calculation processes, and quality-control procedures—must be documented. Packaging and labeling processes also must be recorded. All of these activities would be protected by the safe harbor, even if they otherwise would infringe valid method patents, and even if the otherwise-infringing activities

were used to compete directly with the patentee. This interpretation would "allow almost all activity by pharmaceutical companies to constitute 'submission' and therefore justify a free license to trespass" on patent rights. *Momenta I*, 686 F.3d at 1367 (Rader, C.J., dissenting).

For the same reasons, such an interpretation could extend safe harbor protection beyond method patents. Composition patents are infringed by making or using the composition. The FDA likewise requires the maintenance of records of the components used in the manufacturing process and of the actual formulation of a drug product. 21 C.F.R. §§ 211.180 *et seq*. For example, batch records must document the compounds used in a drug's active pharmaceutical ingredient, compounds added to make the finished drug product, equipment used in manufacturing, packaging and labeling equipment, and the actual packaging used. A12264. Under the district court's rationale, the safe harbor thus could immunize the use of patents on biologic media and media components; on formulation and formulation components; on syringes, pens, and other delivery devices; and on packaging technology.

Momenta I observed that Amphastar's use and its batch records of such use are not "routine" because they "are required to maintain FDA approval." 686 F.3d at 1358. But in that sense, no pharmaceutical manufacturing activity is "routine." The statutes and regulations cited in *Momenta I* are not unique to Amphastar or

enoxaparin; they apply to *all* manufacturing steps, *all* manufacturers, and *all* drugs. *Id.* at 1358 (citing 21 C.F.R. §§ 211.165, 211.180, 211.186, 211.188, 211.194). As a condition of continuing approval, all manufacturers are obligated to conform to the manufacturing procedures that they chose to propose in their NDA or ANDA and that the FDA approved, and all manufacturers must maintain records documenting the use of those procedures. 21 U.S.C. §§ 331(e), 335a(g), 355(e)(5); 21 C.F.R. §§ 211.165(d), 314.70, 314.97.

In short, under the district court's rationale, the safe harbor would apply to any infringing commercial manufacturing activity any time the FDA requires the drug manufacturer to document that use and maintain records for possible FDA inspection. But that is *every* time. There is no indication in the statutory text, purpose, or legislative history that Congress intended for traditional patent law to be upended so dramatically. Indeed, to read the safe harbor so broadly as to encompass ordinary commercial manufacturing activities would discourage innovation in the pharmaceutical sciences. That cannot be what Congress intended. For all of these reasons, summary judgment of non-infringement under Section 271(a) should be reversed.

II. EVEN IF THE SAFE HARBOR EXEMPTS AMPHASTAR'S USE OF MOMENTA'S METHOD, AMPHASTAR'S SALES EFFORTS ARE NOT PROTECTED AND THEY INFRINGE UNDER SECTION 271(g)

Even if the safe harbor protects Amphastar's use of Momenta's patented invention (which otherwise would be infringement under Section 271(a)), summary judgment still should be reversed for an independent reason: Amphastar's use of Momenta's method to manufacture enoxaparin is not its only infringing conduct; Amphastar's sales activity separately infringes under Section 271(g), which makes it an act of infringement to "offer[] to sell" or "sell[] . . . within the United States a product which is made by a process patented in the United States." 35 U.S.C. § 271(g); *see supra* p. 13 n.1. This distinct sales activity, which was not addressed in *Momenta I*, must be analyzed separately to determine whether it is protected by Section 271(e)(1). *Amgen*, 565 F.3d at 852.

While the safe harbor could protect conduct otherwise infringing under Section 271(g) if the conduct fell within the terms of Section 271(e)(1), Amphastar's sales efforts do not. Amphastar's sales efforts are not "solely for uses reasonably related to the development and submission of information under" the FDCA. Nor do they fall within even the broadest possible reading of *Momenta I*. *Momenta I* concluded on the preliminary-injunction record that Amphastar's use likely was exempt based on the understanding that "Amphastar is required by the FDA to use" an infringing procedure and because of requirements in the FDCA

and the FDA's Good Manufacturing Practice regulations that Amphastar "maintain records" documenting that use for possible FDA inspection. 686 F.3d at 1357, 1361. That rationale does not apply to Amphastar's sales efforts: there is no requirement that records of those efforts be submitted *or* maintained.

Those sales efforts infringe under the plain terms of Section 271(g). Amphastar "offers to sell" and "sells . . . within the United States" its enoxaparin. 35 U.S.C. § 271(g); *see* A13051-A13054; A13058-A13062; A13069-A13071; A13288-A13289; A13290-A13300. Momenta's method is a "process patented in the United States." 35 U.S.C. § 271(g). And Amphastar's enoxaparin "is made by" Momenta's process. *Id.* Specifically, Amphastar uses Momenta's method as an intermediate step in the multi-step process of manufacturing its drug, to select the individual batches of interim enoxaparin preparation it will further process into final drug product. A12439-A12440. The FDA's Good Manufacturing Practice regulations include quality-control procedures, such as Momenta's method, as integral parts of drug manufacturing. 21 C.F.R. §§ 210.3(b)(12), 211.100, 211.110(a), (c); A12441. And the pharmaceutical manufacturing industry understands quality-control procedures such as Momenta's to be part of the manufacturing of a drug product. A12440-A12443.

Because "the statute's language is plain, 'the sole function of the courts is to enforce it according to its terms.'" *Ron Pair*, 489 U.S. at 241 (quoting *Caminetti*,

242 U.S. at 485). Amphastar's conduct falls within the terms of Section 271(g), and the district court therefore should have denied summary judgment of non-infringement.

The district court held otherwise only by imposing a limitation not required by the statutory text. The district court interpreted Section 271(g) as requiring "'importation or sale of the product of a patented process *practiced abroad*.'" A10 (emphasis added) (quoting *Cardiac Pacemakers*, 576 F.3d at 1369 (Newman, J., dissenting)). The court held that "[b]ecause there is no suggestion that Amphastar manufactures enoxaparin abroad, § 271(g) is inapplicable." A10-A11.

But the statutory text does not require that Momenta's process be practiced abroad. The only geographic requirements imposed by Section 271(g) are that (1) the process be "patented in the United States" and (2) the sales activity occur "within the United States." 35 U.S.C. § 271(g). Section 271(g) does not impose an additional geographic requirement that the process be practiced abroad and that the product be imported.

Indeed, a plain reading of Section 271(g) makes this clear. The first sentence provides in full: "Whoever without authority imports into the United States *or* offers to sell, sells, or uses within the United States a product which is made by a process patented in the United States shall be liable as an infringer, if the *importation, offer to sell, sale, or use* of the product occurs during the term of

such process patent." *Id.* (emphasis added). The "or" divides two separate liability-creating clauses: infringement occurs by "import[ing] into the United States" "or" by "offer[ing] to sell, sell[ing], or us[ing] within the United States." Nothing in the language or structure of this provision makes foreign manufacture and importation a prerequisite to liability for offering to sell or selling. To the contrary, that there are four independent ways to infringe is confirmed by the second half of the sentence, which gives each separate act equal footing in a single phrase, divided only by commas: "if the importation, offer to sell, sale, or use of the product occurs during the term of such process patent." *Id.*

This reading of Section 271(g) is consistent with its purpose. It is part of a "Congressional scheme . . . to give relief to process patent holders when the resulting products of their patented process are used within the United States—regardless of where the process is practiced." *Zoltek Corp. v. United States*, 672 F.3d 1309, 1315 (Fed. Cir. 2012). Indeed, Congress did not differentiate in Section 271(g) based on the place where the patented process is practiced, so as to comply with non-discrimination obligations under the General Agreement on Tariffs and Trade. S. Rep. No. 100-83, at 46 (1987); H.R. Rep. No. 100-60, at 11 n.42 (1987).

In concluding otherwise, the district court relied on a statement in a dissent from an en banc decision, treating the statement as if it were this Court's holding.

A10 (quoting *Cardiac Pacemakers*, 576 F.3d at 1369 (Newman, J., dissenting)). Moreover, that dissent was not even addressing Section 271(g); it was discussing the scope of Section 271(*f*). *Cardiac Pacemakers*, 576 F.3d at 1369 (Newman, J., dissenting).

The district court likewise erred in relying on "language of § 271(g) which provides that it is only applicable to the infringement of a process patent if 'there is no adequate remedy under this title for infringement.'" A10 (quoting 35 U.S.C. § 271(g)). That limitation applies only where infringement results from a product's "*non*commercial use or *retail* sale." 35 U.S.C. § 271(g) (emphasis added). That is not the case here: it is undisputed that Amphastar has offered for sale and sold [redacted] enoxaparin for commercial purposes to wholesalers, group purchasing organizations, hospitals, and pharmacies. A13050-A13073; A13288-A13300. And in any event, under Amphastar's view of the safe harbor, there is no adequate remedy provided elsewhere in the patent laws.

Accordingly, regardless of whether Amphastar's use is exempted by the safe harbor, summary judgment of non-infringement still should be reversed because Amphastar's offers to sell and sales infringe under Section 271(g).

III. IT WOULD NOT HAVE BEEN FUTILE FOR MOMENTA TO AMEND ITS INFRINGEMENT CONTENTIONS

The sole basis for the district court's ruling denying Momenta's motion for leave to amend its infringement contentions was its conclusion that "the proposed amendments would be futile." A12. The district court ruled that its "summary judgment holding that the 271(e)(1) safe harbor provision applies to the 15-25% procedures also applies to the DBB test." A12. And the court held that batch-to-batch comparisons do not infringe because the court was "skeptical that the so called batch-to-batch test is even a separate testing procedure." A13. These futility conclusions were legally erroneous.

1. Accusing Amphastar's DBB Procedure and its batch-to-batch comparisons would not have been futile

As an initial matter, the district court's conclusion was based on the faulty premise that Amphastar's 15-25% 1,6-anhydro procedure is protected by the safe harbor and thus does not infringe. But if summary judgment as to the 15-25% 1,6-anhydro procedure is reversed on any basis, the denial of Momenta's motion for leave also should be reversed. For the same reasons that Amphastar's use of the 15-25% 1,6-anhydro procedure is not protected, Amphastar's performances of the DBB Procedure and batch-to-batch comparisons also are outside the safe harbor. And for the same reasons that Amphastar's sales activity with respect to products made by its 15-25% 1,6-anhydro procedure infringes under Section 271(g),

Amphastar's sales activity with respect to enoxaparin that has been made by use of the DBB Procedure and batch-to-batch comparisons also infringes.

But even if summary judgment is affirmed with respect to Amphastar's 15-25% 1,6-anhydro procedure, the denial of Momenta's motion for leave still should be reversed. [redacted] And Amphastar's batch-to-batch comparisons are used to determine whether there are any untoward trends in manufacturing, to make adjustments, and to ensure consistency in drug product. A12356, A12377, A12395.

2. At the very least, accusing Amphastar's batch-to-batch comparisons would not have been futile

In any event, and at the very least, Amphastar's batch-to-batch comparisons are not protected by the safe harbor, even under the broadest possible reading of *Momenta I*. There is no evidence that Amphastar proposed batch-to-batch comparisons as part of its manufacturing process or that the FDA approved these

comparisons. Nor has Amphastar produced in discovery any documentary evidence that it submits to the FDA or even maintains records of batch-to-batch comparisons.

The district court nevertheless believed that the batch-to-batch comparison could not infringe because it was "skeptical" that it is "a separate testing procedure" from the procedures that infringe other claims of the '886 patent. A13. That is not the correct legal inquiry. Claim 1 is a separate claim. If Amphastar performs all steps of claim 1, it infringes that claim, regardless of whether it infringes other claims. *Joy Techs., Inc. v. Flakt, Inc.*, 6 F.3d 770, 773 (Fed. Cir. 1993). This "accused activit[y] must be evaluated separately to determine whether the [Section 271(e)(1)] exemption applies." *Amgen*, 565 F.3d at 852.

And Amphastar's batch-to-batch comparisons practice claim 1. The first three steps of claim 1 are essentially the same as the first three steps of the other asserted claims, claims 6 and 53. These steps involve performing exhaustive digestion, using a separation method, and comparing the results against a reference standard, all of which Amphastar performs. A102(col.63:56-col.64:2). The last step of claim 1 is different from the other claims and recites: "determining the presence of the structural signature . . . in a second batch of enoxaparin." A102(col.64:3-5). Amphastar performs that final step—and thus infringes claim 1—when it performs either its 15-25% Procedure or its DBB Procedure on

two batches of enoxaparin and compares the results. A12376-A12377, A12394-A12395.

Accordingly, the district court legally erred in concluding that an amendment would be futile.

CONCLUSION

Summary judgment of non-infringement should be reversed, denial of leave to amend the infringement contentions should be reversed, and the case should be remanded for further proceedings.

Respectfully submitted,

JUNE 27, 2014

s/ Deanne E. Maynard

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ADDENDUM

United States District Court
District of Massachusetts

MOMENTA PHARMACEUTICALS, INC. AND SANDOZ INC.,	)	
Plaintiffs,	)	
v.	)	**Civil Action No. 11-11681-NMG**
AMPHASTAR PHARMACEUTICALS, INC., INTERNATIONAL MEDICATION SYSTEMS, LTD., ACTAVIS, INC. AND WATSON PHARMA, INC.,	)	
Defendants.	)	

ORDER OF FINAL JUDGMENT

In accordance with the Court's Order dated July 19, 2013 allowing defendants' Motion for Summary Judgment in the above-titled action, it is hereby **ORDERED:**

1) Judgment shall enter in defendants' favor and against plaintiffs on Counts I and III of plaintiffs' Amended Complaint (Docket No. 63);

2) Judgment shall enter in defendants' favor and against plaintiffs on the First Counterclaim in defendants' Amended Answer to plaintiffs' Amended Complaint (Docket No. 221) to the extent defendants seek declaratory judgment of non-infringement of U.S. Patent No. 7,575,886; and

3) Counts II and IV of plaintiffs' Amended Complaint, defendants' First Counterclaim to the extent defendants seek declaratory judgment of non-infringement of U.S. Patent No. 7,790,466, and defendants' Second Counterclaim, are dismissed without prejudice.

Dated January 24, 2014

/s/ Nathaniel M. Gorton
Nathaniel M. Gorton
United States District Judge

United States District Court
District of Massachusetts

MOMENTA PHARMACEUTICALS, INC., SANDOZ INC., Plaintiffs,	)	
v.	)	**Civil Action No. 11-11681-NMG**
AMPHASTAR PHARMACEUTICALS, INC., INTERNATIONAL MEDICATION SYSTEMS, LTD., WATSON PHARMACEUTICALS, INC., Defendants.	)	

MEMORANDUM & ORDER

GORTON, J.

Plaintiffs Momenta Pharmaceuticals, Inc. ("Momenta") and Sandoz Inc. ("Sandoz") (collectively, and for simplicity, "Momenta") bring suit against Amphastar Pharmaceuticals, Inc. ("Amphastar"), International Medication Systems, Ltd., Actavis, and Watson Pharma, Inc. (collectively, and for simplicity, "Amphastar") for infringement of U.S. Patent No. 7,575,886 ("the '886 patent") and declaratory judgment of infringement.[1]

[1] Momenta originally also asserted infringement of U.S. Patent No. 7,790,466 ("the '466 patent") but indicated in its opposition to defendants' motion for summary judgment that it is withdrawing that claim. Thus, this Court need not address that claim.

I. Background

The facts of this case have previously been extensively described both by this Court and by the United States Court of Appeals for the Federal Circuit and need not be repeated at length here. In brief, in July, 2010, after receiving FDA approval, plaintiffs began to market the first generic version of Lovenox (otherwise known as enoxaparin) in the United States. Enoxaparin is an anticoagulant used to prevent blood clots. Amphastar received FDA approval to market its generic enoxaparin product on September 19, 2011.

Momenta is the assignee of the ’886 patent, issued in August, 2009, which is directed at a set of manufacturing control processes that ensure that each batch of generic enoxaparin includes the individual sugar chains characteristic of Lovenox. Momenta alleges that Amphastar infringes the ‘886 patent by manufacturing generic enoxaparin for commercial sale using the claimed methods of the patent.

II. Procedural History

Plaintiffs filed the instant action on September 21, 2011, two days after Amphastar received FDA-approval of its generic enoxaparin product. Shortly thereafter, plaintiffs moved for a temporary restraining order and preliminary injunction to prevent Amphastar from marketing its product, which the Court allowed. Defendants appealed that ruling to Federal Circuit. On January

25, 2012, the Federal Circuit stayed the preliminary injunction pending appeal.

This Court held a joint Markman hearing in this case and Momenta Pharm. Inc, v. Teva Pharm., C.A. No. 11-cv-12079-NMG, in May, 2012, and issued a Markman Order in June, 2012. On August 3, 2012, the Federal Circuit vacated the preliminary injunction. Shortly thereafter, on August 14, 2012, at the request of the parties, this Court stayed the case pending an en banc appeal in the Federal Circuit. In November, 2012, the Federal Circuit denied the petition for an en banc hearing. Amphastar then filed a motion to remove the stay. This Court delayed ruling on that motion due to a petition for certiorari to the Supreme Court in Classen Immunotherapies, Inc. v. Biogen IDEC, 659 F.3d 1303 (Fed Cir. 2011), which also raised issues relating to the so called "safe-harbor" under 35 U.S.C. § 271(e)(1) ("§ 271(e)(1)"). After the Supreme Court denied cert in Classen, this Court lifted the stay in this case on January 15, 2013.

On January 16, 2013, Amphastar moved for Summary Judgment and Judgment on the Pleadings. Recently, Momenta requested leave to amend its infringement contentions and on July 1, 2013 the Court heard oral argument on both motions and took the matter under advisement. The Court now announces its ruling on both motions.

III. Federal Circuit Decision

In overturning this Court's determination that Momenta had proven a likelihood of success on the merits sufficient to warrant a preliminary injunction, the Federal Circuit ruled that the "safe harbor" provision of § 271(e)(1) applied to this case. That provision states that

> It shall not be an act of infringement to make, use, offer to sell, or sell within the United States or import into the United States a patented invention . . . solely for uses reasonably related to the development and submission of information under a Federal law which regulates the manufacture, use, or sale of drugs or veterinary biological products.

In interpreting § 271(e)(1), the Federal Circuit explained that Congress broadly defined the scope of the safe harbor and thus the protection provided by the safe harbor is not limited to "activities necessary to seek approval of a generic drug", but rather encompasses all "materials the FDA demands in the regulatory process." Momenta Pharm. v. Amphastar Pharm., 686 F.3d 1348, 1356 (2012). Therefore, the Federal Circuit determined that even post-FDA approval activities are covered by the safe harbor, as long as they are "reasonably related to the development and submission of information under a Federal law which regulates the manufacture, use or sale of drugs." Id. at 1358-60.

Citing the requirements in 21 C.F.R. § 211.180(a) that testing records from each batch of generic enoxoparin must be

"retained for at least 1 year after the expiration date of the batch" and in 21 C.F.R. § 211.180(c) that those records "shall be readily available for authorized inspection" by the FDA, the Federal Circuit held that the requirement to maintain records for FDA inspection satisfies the "requirement that the uses be reasonably related to the development and submission of information to the FDA." The Federal Circuit also held that "the fact that the FDA does not in most cases actually inspect the records does not change" that reasoning. Momenta, 686 F.3d at 1357 (citing § 271(e)(1)).

In light of it's decision the Federal Circuit instructed this Court to consider on remand

> whether Momenta's admission that Amphastar's use of the patented invention is to 'satisfy the FDA's requirements' makes this case amenable to summary judgment of non-infringement in favor of Amphastar.

Momenta Pharma. v. Amphastar Pharma., 686 F.3d 1348, 1361 (Fed. Cir. 2012).

IV. Motion for Summary Judgment[2]

A. Standard

The role of summary judgment is "to pierce the pleadings and to assess the proof in order to see whether there is a genuine

[2] Although defendants filed a motion for Judgment on the Pleadings and Summary Judgment, in light of the fact that the Federal Circuit requested that this Court consider whether the case is "amenable to summary judgment of non-infringement", and the fact that the outcome would be the same, the Court treats the motion as one for Summary Judgment.

need for trial." Mesnick v. Gen. Elec. Co., 950 F.2d 816, 822 (1st Cir. 1991) (quoting Garside v. Osco Drug, Inc., 895 F.2d 46, 50 (1st Cir. 1990)). The burden is on the moving party to show, through the pleadings, discovery and affidavits, "that there is no genuine issue as to any material fact and that the moving party is entitled to judgment as a matter of law." Fed. R. Civ. P. 56(c).

A fact is material if it "might affect the outcome of the suit under the governing law." Anderson v. Liberty Lobby, Inc., 477 U.S. 242, 248 (1986). "Factual disputes that are irrelevant or unnecessary will not be counted." Id. A genuine issue of material fact exists where the evidence with respect to the material fact in dispute "is such that a reasonable jury could return a verdict for the nonmoving party." Id.

Once the moving party has satisfied its burden, the burden shifts to the non-moving party to set forth specific facts showing that there is a genuine, triable issue. Celotex Corp. v. Catrett, 477 U.S. 317, 324 (1986). The Court must view the entire record in the light most favorable to the non-moving party and make all reasonable inferences in that party's favor. O'Connor v. Steeves, 994 F.2d 905, 907 (1st Cir. 1993). Summary judgment is appropriate if, after viewing the record in the non-moving party's favor, the Court determines that no genuine issue of material fact exists and that the moving party is entitled to

judgment as a matter of law.

B. Application

i. 35 U.S.C. § 271(e)(1)

In their Complaint plaintiffs alleges that Amphastar must be infringing the '886 patent because the Food and Drug Administration ("FDA") requires Amphastar to perform the methods claimed in the patent. Defendants move for summary judgment on the grounds that all of their allegedly infringing activity is subject to the § 271(e)(1) safe harbor and thus cannot constitute patent infringement. Plaintiffs oppose on several grounds.

First, plaintiffs argue that summary judgment is not appropriate because the safe harbor does not apply if the FDA has not mandated the use of the particular infringing test. Momenta contends that "Amphastar's use of Momenta's patented process was entirely voluntary" because the FDA has not specifically required Amphastar to use the test covered by the '866 patent. This argument is unavailing. There is no language in § 271(e)(1) that limits the application of the safe harbor to situations in which the FDA has expressly required an applicant to use a particular infringing test. Instead, the Federal Circuit construed the safe harbor such that it provides a "wide berth." Id. at 1356. Thus,

> as long as the use of the patented invention is done to generate information that will be submitted pursuant to a relevant federal law, that use falls within the safe harbor.

Id.

Furthermore, the Federal Circuit explicitly held that the safe harbor "does not mandate the use of a noninfringing alternative when one exists." Momenta, 686 F.3d at 1359. The Court further noted that Momenta is

> incorrect that the possibility that the FDA would accept the use of other, non-patented, testing methods for the development and submission of information precludes Amphastar from relying on the safe harbor in this case.

Id. at 60. Moreover, if the safe harbor covered only infringing tests that are required by the FDA, it would be in conflict with the Supreme Court's holding in Merck v. Integra Lifesciences I, LTD, 545 U.S. 193 (2005). In that case the Court held that pre-filing tests that were not ultimately submitted to the FDA were still covered by the safe harbor because such pre-filing tests could never have been required by the FDA.

Second, plaintiffs argue that Amphastar's "[r]outine, post-approval recordkeeping" is not "submission of information" to the FDA because Amphastar does not actually "submit" these results and thus such maintenance is not covered by the safe harbor. Plaintiffs cite numerous dictionary definitions to attempt to distinguish "submission" from mere "maintenance". These definitions do not, however, negate the fact that the Federal Circuit expressly held that the maintenance of records for FDA inspection "satisfies the requirement that the uses be reasonably related to the development and submission of information to the

FDA." Id. at 1357 (emphasis added). The Court further noted that "the fact that the FDA does not in most cases actually inspect the records does not change" the fact that the records are reasonably related to "submission" of information to the FDA. Id. (citing Merck, 545 U.S. 193 at 207).

Momenta also avers that Amphastar's alleged use of the patented method during manufacturing "so that it can sell [enoxaparin] and earn profit" makes that use not "solely" for "uses reasonably related to the development and submission of information" to the FDA. Plaintiffs assert that Amphastar's routine commercial manufacturing conducted "long after FDA approval" therefore makes that use "well beyond" the reach of the safe harbor. Unfortunately for plaintiffs, the Federal Circuit found that such an argument is "not a tenable reading of the statute" and is "contrary to precedent." Id. at 1360. For example, the Federal Circuit has previously held that "alternate uses [of test data] are irrelevant to [the] qualification to invoke the section 271(e)(1) shield" because the safe harbor allows alleged infringers to use test data for "more than FDA approval." Abtox, Inc. v. Exitron Corp., 122 F.3d 1019, 1030 (Fed. Cir. 1997). Defendants' activities are thus protected by the safe harbor.

ii. 35 U.S.C. § 271(g)

Momenta also asserts that summary judgment of non-

infringement is inappropriate on the ground that defendants are conducting infringing activity under 35 U.S.C. § 271(g). That statute provides, in relevant part, that:

> Whoever without authority imports into the United States or offers to sell, sells, or uses within the United States a product which is made by a process patented in the United States shall be liable as an infringer.

Plaintiffs argue that defendants are "liable as...infringer[s]" because they offer to sell and sell a product made by a process patented in the United States.

Plaintiffs rely on the plain language of 35 U.S.C. § 271(g) to contend that the statute "makes no distinction between the use of a patented process inside or outside the United States." Such an argument ignores the fact that the Federal Circuit has explicitly stated that § 271(g)

> requires importation or sale of the product of a patented process practiced abroad, before infringement can be established under that provision.

Cardiac Pacemakers, Inc. v. St. Jude Med., Inc., 576 F.3d 1348, 1369 (Fed. Cir. 2009). That reasoning is supported by the language of § 271(g) which provides that it is only applicable to the infringement of a process patent if "there is no adequate remedy under this title for infringement." Section 271(a) applies to the making or use of a patented invention within the United States, and therefore § 271(g) would not apply in those circumstances. Because there is no suggestion that Amphastar manufactures enoxaparin abroad, § 271(g) is inapplicable in this

case. As a result, Amphastar cannot be liable for infringement pursuant to § 271(g).

V. Motion to Amend Infringement Contentions

In their preliminary Infringement Contentions served on February 7, 2012, plaintiffs accused two of Amphastar's procedures: the "Approved 15-25% Procedure" which Amphastar performed at the time of FDA approval, and its "Revised 15-25% Procedure" which it adopted after FDA approval. In its Amended Infringement Contentions served on February 12, 2013, Momenta accuses two additional Amphastar procedures. The first is the Disaccharide Building Block Procedure ("DBB test"). The DBB test is the same as the two 15-25% procedures except that it compares the presence and amount of particular digested sub-chains to individual reference standards for those specific sub-chains rather than to the 15-25% reference standard. The second test plaintiffs seek to add is the "Batch-to-Batch" procedure. This method seems to involve a simple comparison between the results obtained through one of the other three tests on a particular batch of enoxaparin and the results obtained on another batch.

In its Second Amended Infringement Contentions served on May 24, 2013, Momenta added further documentary support for the newly added infringement contentions. Momenta now seeks leave from the Court for both its Amended and Second Amended Infringement Contentions having failed to seek leave prior to serving them as

required by the scheduling order in this case (Docket No. 139).

A. Standard

A scheduling order may be modified only for good cause and with the judge's consent. Fed. R. Civ. P. 16(b)(4). In determining whether to grant leave to amend, a court generally considers 1) the explanation for the failure to move timely for leave to amend 2) the importance of the amendment 3) the potential for prejudice caused by allowing the amendment and 4) the opportunity to cure such prejudice. E.g. S&W Enterprises, L.L.C. v. SouthTrust Bank of Alabama, NA, 315 F.3d 533, 536 (5th Cir. 2003).

B. Application

The Court is concerned by Momenta's allegations that the documents necessary to discover the additional tests were intentionally concealed by defendants, possibly in violation of a court order. Despite those concerns, however, the Court will deny the motion to amend because the proposed amendments would be futile in any event.

First, the reasoning of this Court's summary judgment holding that the 271(e)(1) safe harbor provision applies to the 15-25% procedures also applies to the DBB test. Despite plaintiffs contention that the "FDA has no requirement that Amphastar use a method that infringes the '886 patent" the FDA did actually require defendants to perform the DBB test as part

of their ANDA application, in addition to performing the 15-25% tests. Thus, such testing as required by the FDA cannot constitute infringement. Any post-approval DBB testing is also covered by the safe harbor because, as explained by the Federal Circuit, the resulting maintenance of test records for FDA inspection "satisfies the requirement that the uses be reasonably related to the development and submission of information to the FDA." Momenta, 686 F.3d at 1357.

Second, plaintiffs' proposed amendment to add the batch-to-batch test would also be futile. Plaintiffs assert that because no records are kept, and thus there is no possible submission of information to the FDA, the batch-to-batch test does not qualify for the 271(e)(1) safe harbor and as such assert that the amendment would not be futile.

This Court is, however, skeptical that the so called batch-to-batch test is even a separate testing procedure. It apparently involves a simple comparison of results of previously conducted release tests across multiple batches of enoxaparin to identify trends. No records are created precisely because no additional testing is conducted. Because the "test" simply involves comparing data that has already been produced it cannot possibly require repeating all of the steps of the '866 patent that would be required for infringement. E.g. EMI Group N. Am., Inc. v. Intel Corp., 157 F.3d 887, 896 (Fed.Cir.1998)("For

infringement of a process invention, all of the steps of the process must be performed, either as claimed or by an equivalent step.") As a result, a mere comparison of already produced data could not possibly infringe the '866 patent.

Plaintiffs argue that the declaration of their expert Dr. Jian Liu provides "a detailed, step-by-step explanation of why the Batch-to-Batch Procedure infringes claim 1 of the '866 patent." Yet, his declaration states only that Amphastar conducts a "batch to batch comparison of its release test results." Noticeably Dr. Liu does not even refer to that step as a separate "batch-to-batch test" nor does he distinguish it as a separate test rather than a procedure conducted following the 15-25% analysis.

Finally, it is illogical to suggest that conducting the original release tests is not an act of infringement due to the safe harbor but simply looking at the data produced by those tests is somehow an act of infringement. Therefore, plaintiffs proposed amendment would be futile and the motion will be denied.

ORDER

In accordance with the foregoing,

1) Defendants' Motion for Summary Judgment (Docket No. 346) is **ALLOWED**, and

2) Plaintiffs' Motion for Leave to Amend Infringement Contentions (Docket No. 456) is **DENIED.**

So ordered.

/s/ Nathaniel M. Gorton
Nathaniel M. Gorton
United States District Judge

Dated July 19, 2013

US007575886B2

(12) **United States Patent**
Venkataraman et al.

(10) **Patent No.: US 7,575,886 B2**
(45) **Date of Patent: Aug. 18, 2009**

(54) **ANALYSIS OF SULFATED POLYSACCHARIDES**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 779 days.

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(58) **Field of Classification Search** .... None
See application file for complete search history.

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(57) **ABSTRACT**

The invention relates to methods and products associated with analyzing and monitoring heterogeneous populations of sulfated polysaccharides. In particular therapeutic heparin products including low molecular weight heparin products and methods of analyzing and monitoring these products are described.

69 Claims, 12 Drawing Sheets

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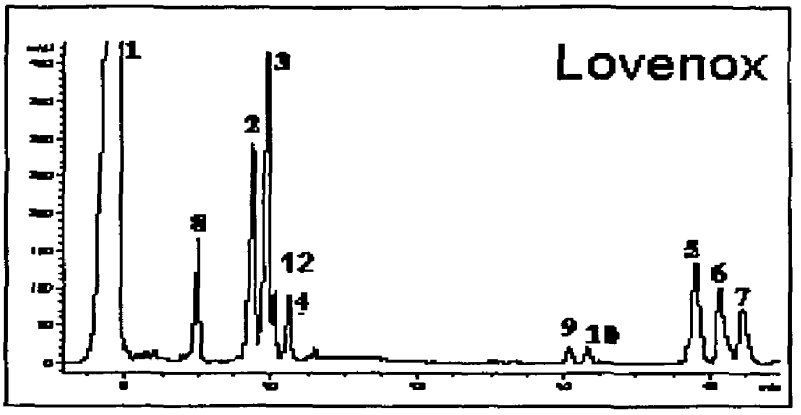
* cited by examiner

Figures 1A-1B

1A.



1B.

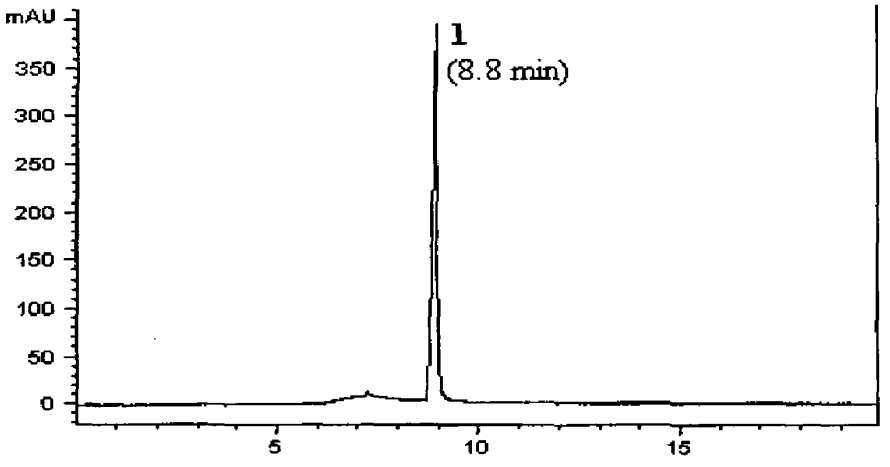


Figure 2

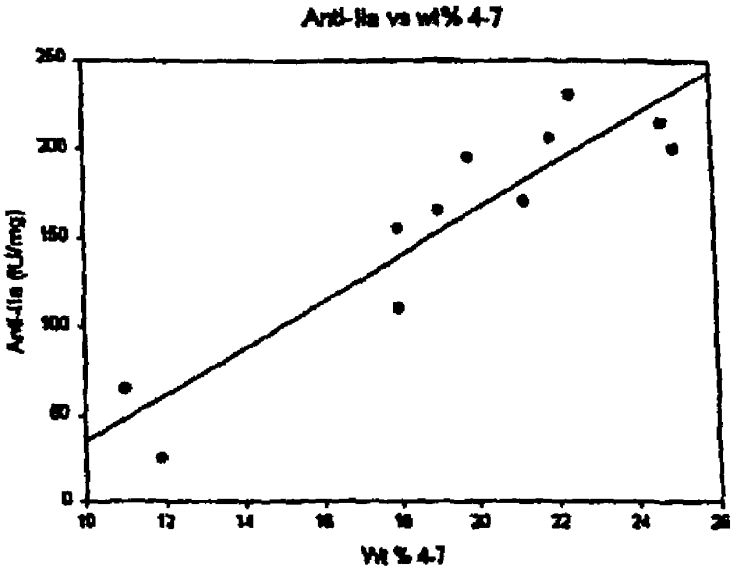


Figure 2A

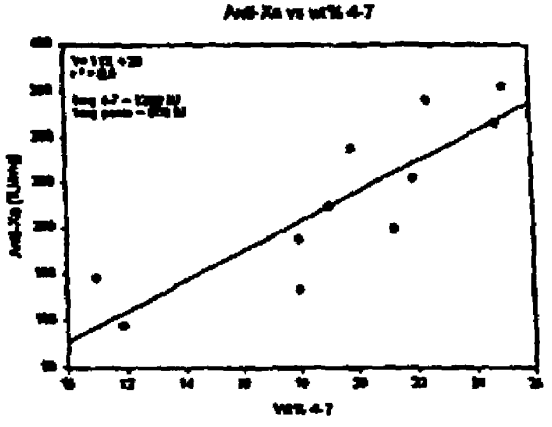
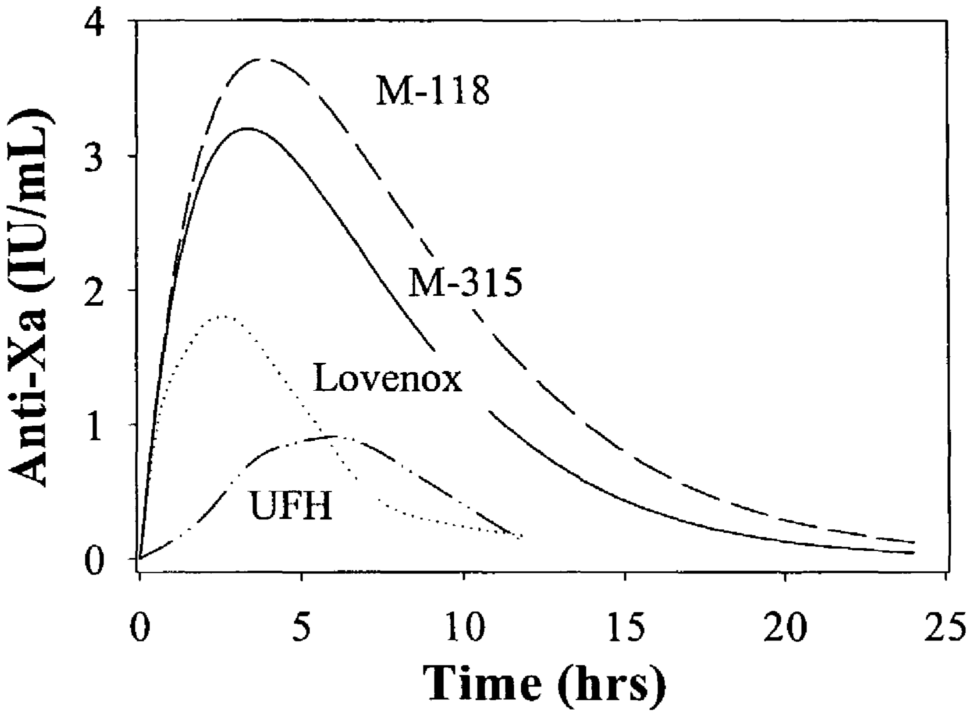


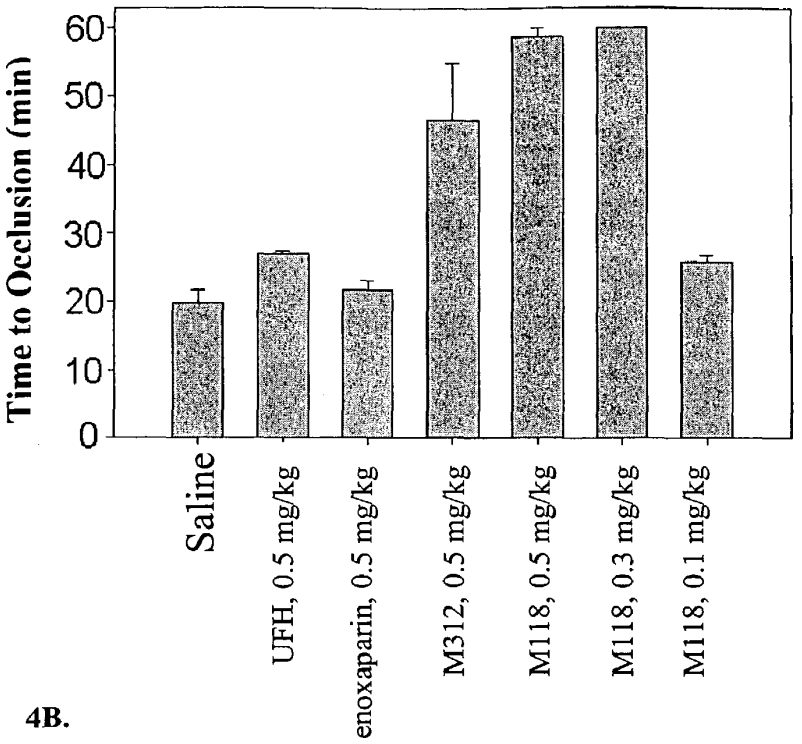
Figure 2B

Figure 3.


4
3
2
1
0
Anti-Xa (IU/mL)
M-118
M-315
Lovenox
UFH
0
5
10
15
20
25
Time (hrs)

Figures 4A-4B.

4A.



4B.

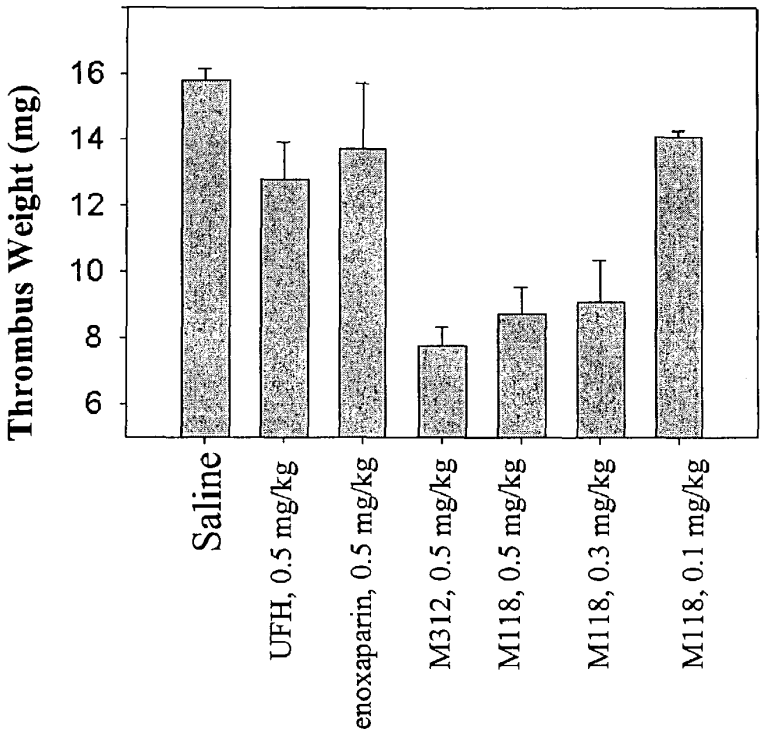


Figure 5.

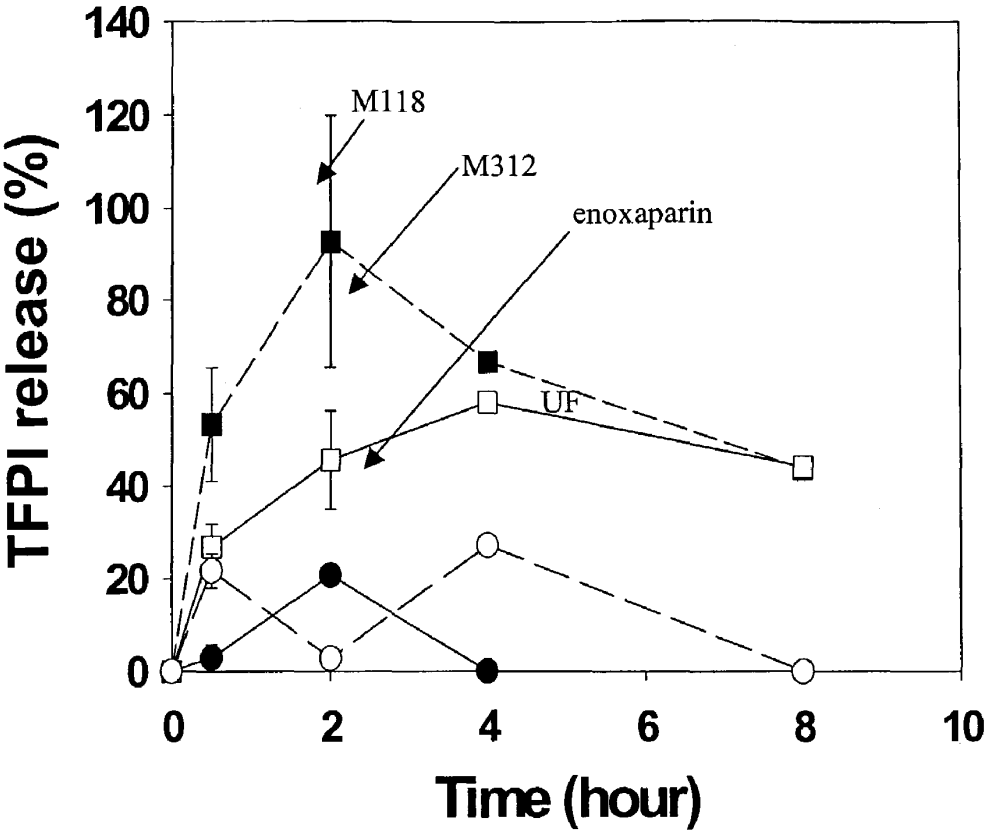

TFPI release (%)
Time (hour)
M118
M312
enoxaparin
UF
0
20
40
60
80
100
120
140
0
2
4
6
8
10

Figure 6.

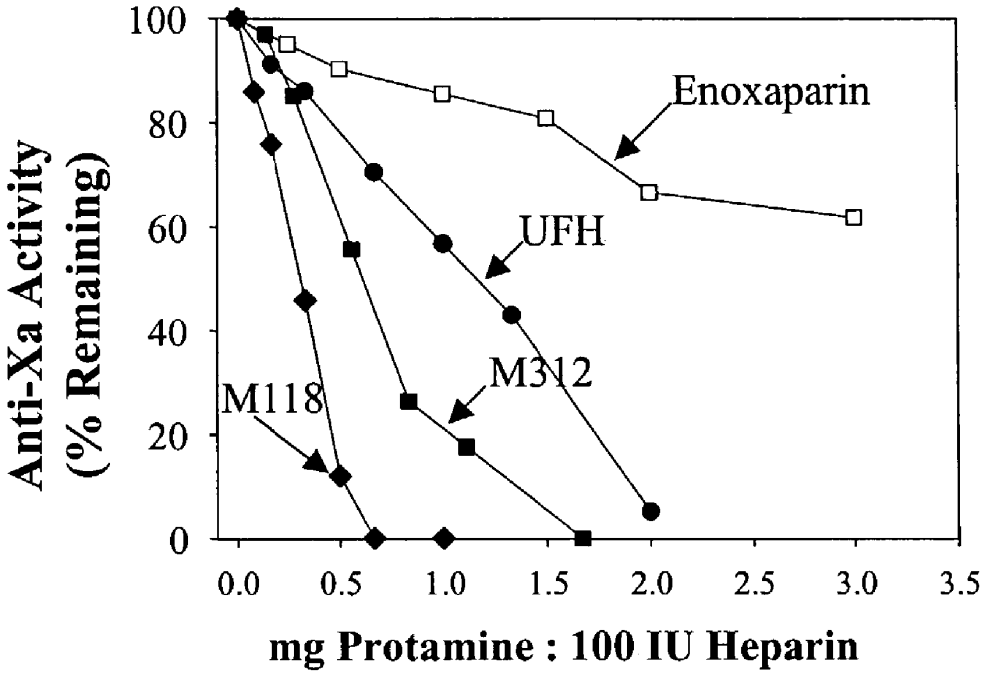

Anti-Xa Activity
(% Remaining)
100
80
60
40
20
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Enoxaparin
UFH
M312
M118
mg Protamine : 100 IU Heparin

Figure 7.

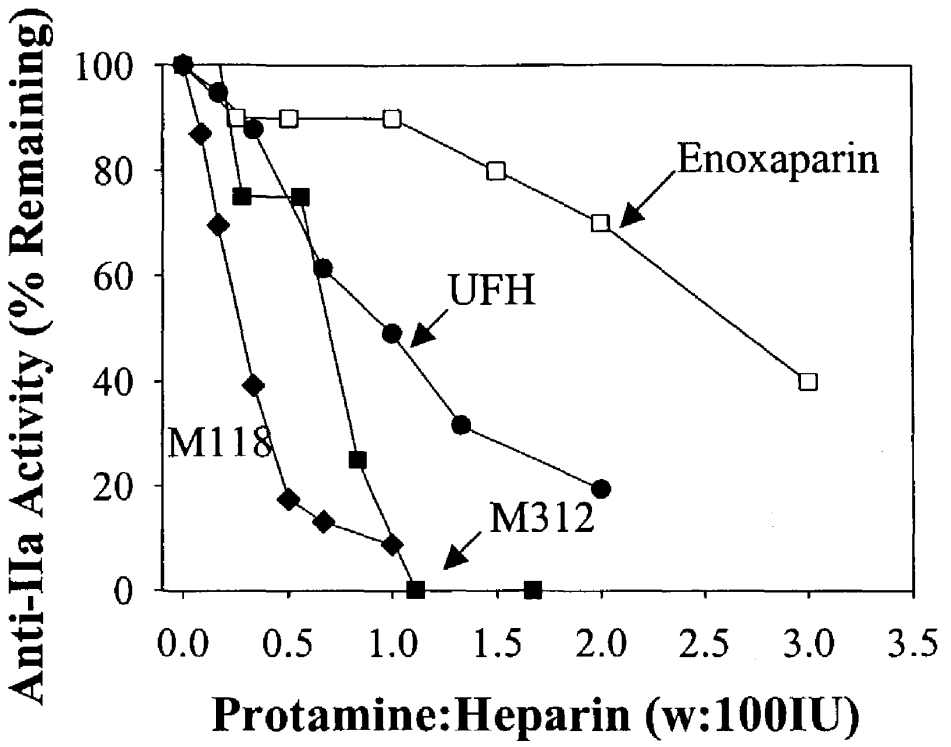

Anti-IIa Activity (% Remaining)
100
80
60
40
20
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Protamine:Heparin (w:100IU)
Enoxaparin
UFH
M118
M312

Figure 8.

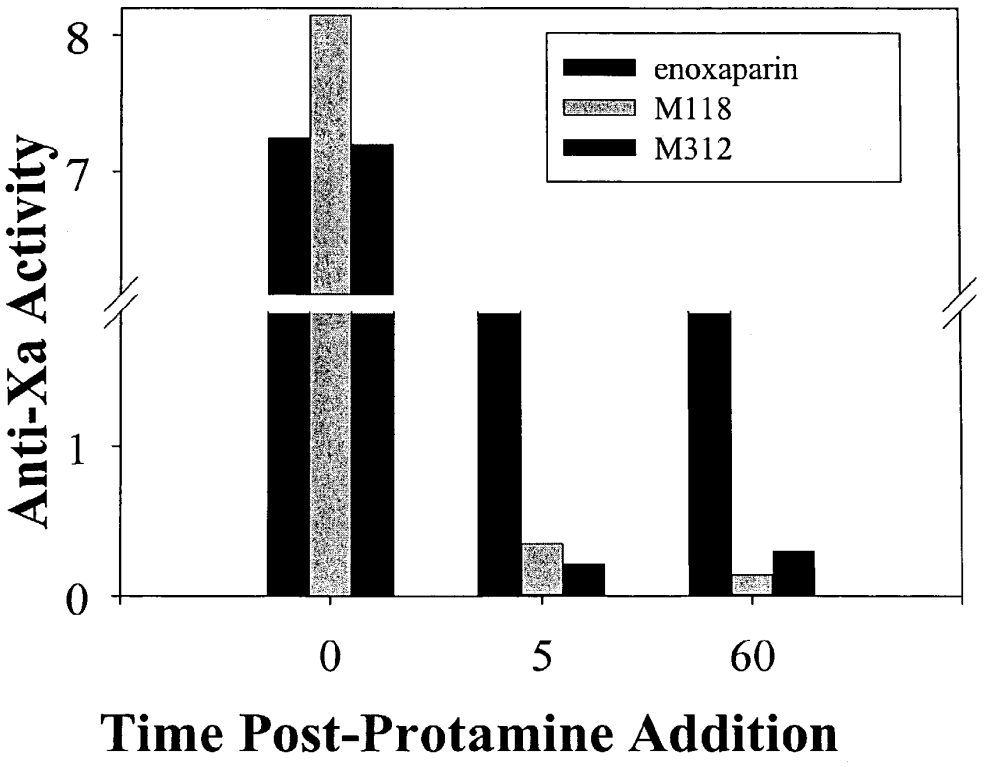
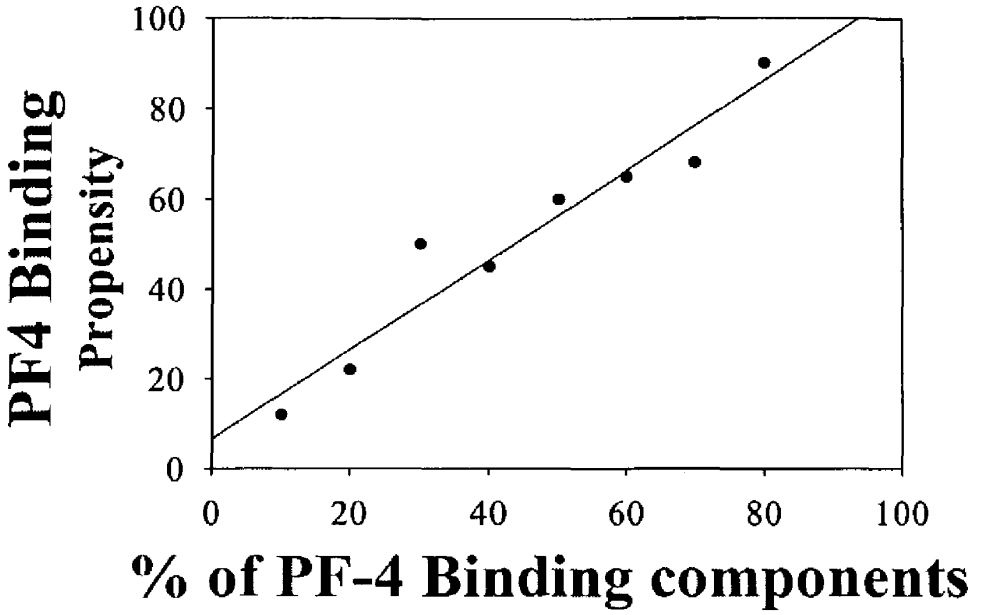
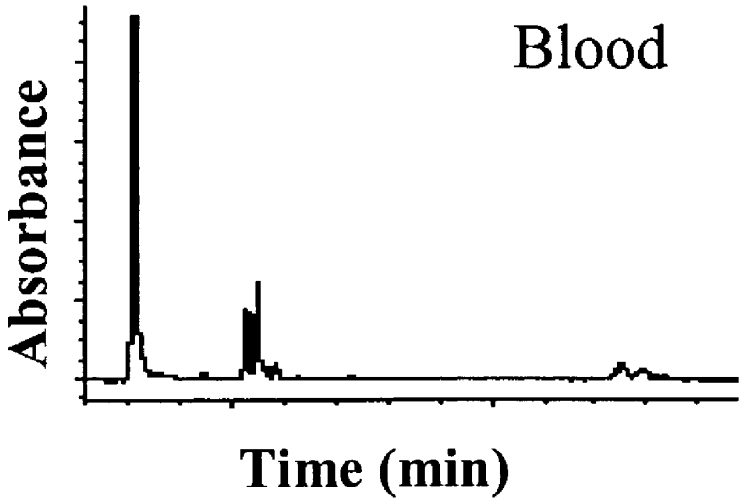

8
7
1
0
Anti-Xa Activity
enoxaparin
M118
M312
0
5
60
Time Post-Protamine Addition

Figure 9.


PF4 Binding
Propensity
100
80
60
40
20
0
0
20
40
60
80
100
% of PF-4 Binding components

Figures 10A-10B.

10A.



10B.

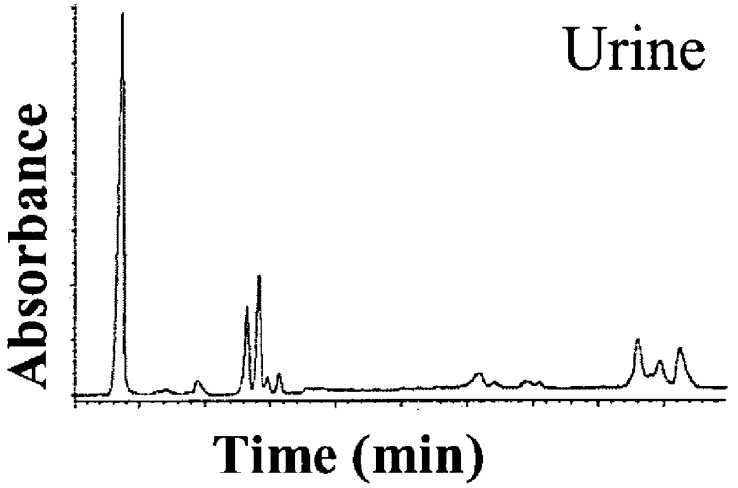
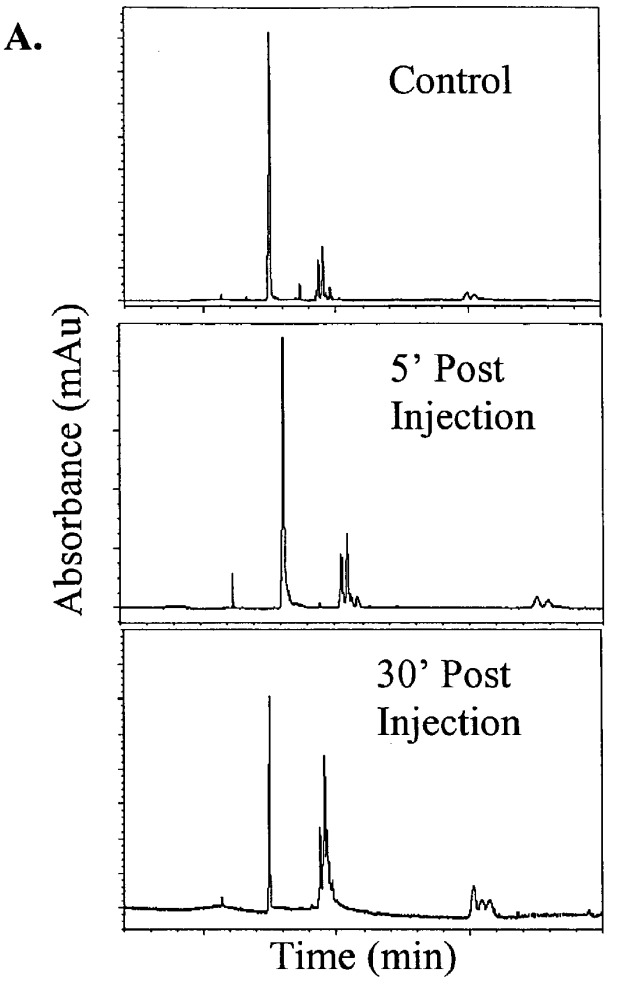


Figure 11.


A.
Control
5' Post Injection
30' Post Injection
Absorbance (mAu)
Time (min)

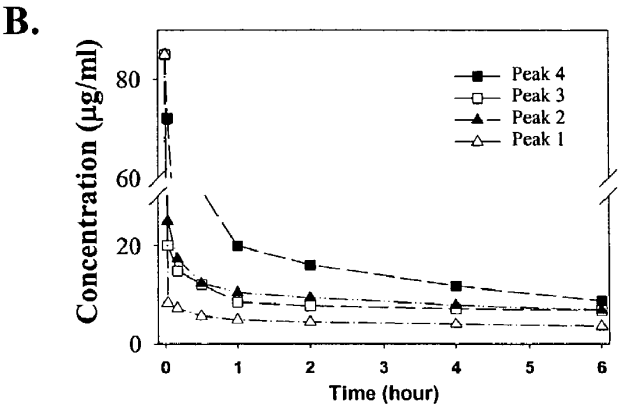
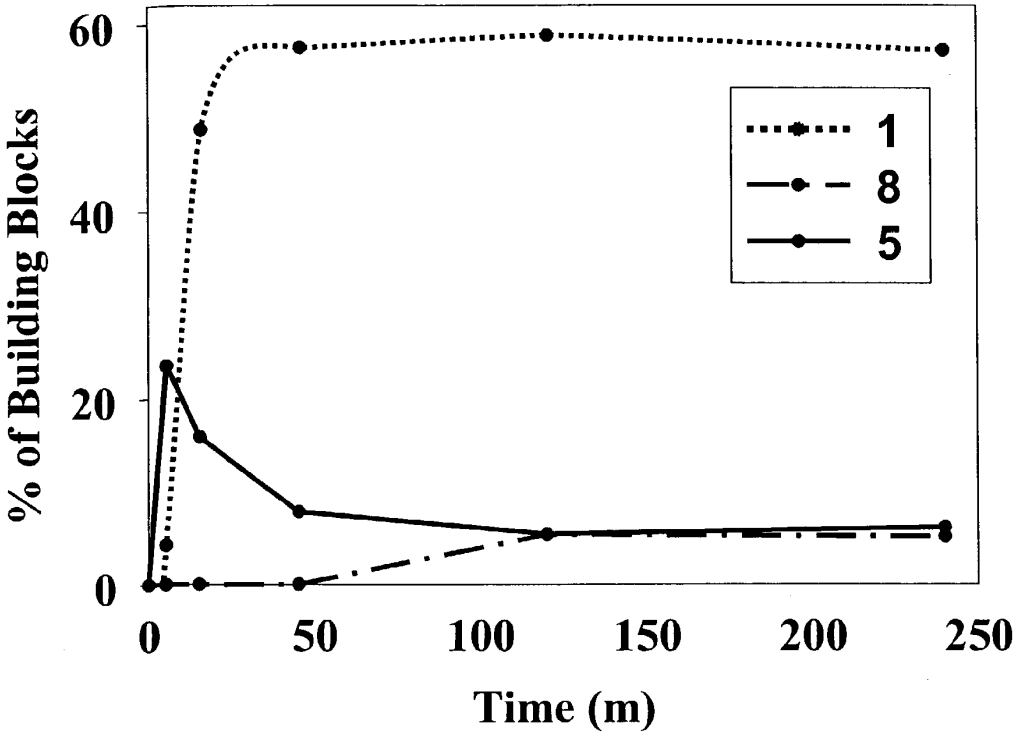

B.
Concentration (μg/ml)
80
60
20
0
Peak 4
Peak 3
Peak 2
Peak 1
0
1
2
3
4
5
6
Time (hour)

Figure 12.



ANALYSIS OF SULFATED POLYSACCHARIDES

CLAIM OF PRIORITY

This application claims priority under 35 USC §119(e) to U.S. Provisional Patent Application Ser. No. 60/393,973, filed on Jul. 5, 2002, U.S. Provisional Patent Application Ser. No. 60/383,903, filed on May 28, 2002, and U.S. Provisional Patent Application Ser. No. 60/363,240, filed on Mar. 11, 2002, the entire contents of which are hereby incorporated by reference.

FIELD OF THE INVENTION

The invention relates to methods and products associated with analyzing and monitoring heterogeneous populations of sulfated polysaccharides. In particular, therapeutic heparin products including low molecular weight heparin products and methods of analyzing and monitoring these products are described.

BACKGROUND OF THE INVENTION

Coagulation is a physiological pathway involved in maintaining normal blood hemostasis in mammals. Under conditions in which a vascular injury occurs, the coagulation pathway is stimulated to form a blood clot to prevent the loss of blood. Immediately after the vascular injury occurs, blood platelets begin to aggregate at the site of injury forming a physical plug to stop the leakage. In addition, the injured vessel undergoes vasoconstriction to reduce the blood flow to the area and fibrin begins to aggregate forming an insoluble network or clot, which covers the ruptured area.

When an imbalance in the coagulation pathway shifts towards excessive coagulation, the result is the development of thrombotic tendencies, which are often manifested as heart attacks, strokes, deep vein thrombosis, myocardial infarcts, unstable angina and acute coronary syndromes. Furthermore, an embolism can break off from a thrombus and result in a pulmonary embolism or cerebral vascular embolism including stroke or transient ischemia attack. Current therapies for treating disorders associated with imbalances in the coagulation pathway involve many risks and must be carefully controlled.

Heparin and low molecular weight heparins (LMWHs), complex, sulfated polysaccharides isolated from endogenous sources, are potent modulators of hemostasis. Heparin, a highly sulfated heparin-like glycosaminoglycan (HLGAG) produced by mast cells, is a widely used clinical anticoagulant, and is one of the first biopolymeric drugs and one of the few carbohydrate drugs. Heparin and molecules derived from it are potent anticoagulants that are used in a variety of clinical situations, especially for thromboembolic disorders including the prophylaxis and treatment of deep venous thrombosis and pulmonary embolism, arterial thromboses, and acute coronary syndromes like myocardial infarction and unstable angina. Heparin and LMWHs interact with multiple components of the coagulation cascade to inhibit the clotting process. Heparin primarily elicits its effect through two mechanisms, both of which involve binding of antithrombin III (AT-III) to a specific pentasaccharide sequence, $H_{NAc/S,6S}GH_{NS,3S,6S}I_{2S}H_{NS,6S}$ contained within the polymer. First, AT-III binding to the pentasaccharide induces a conformational change in the protein that mediates its inhibition of factor Xa. Second, thrombin (factor IIa) also binds to heparin at a site proximate to the pentasaccharide/AT-III binding site.

Formation of a ternary complex between AT-III, thrombin and heparin results in inactivation of thrombin. Unlike its anti-Xa activity that requires only the AT-III pentasaccharide-binding site, heparin's anti-III activity is size-dependent, requiring 1-13 saccharide units in addition to the pentasaccharide unit responsible for anti-Xa activity for the efficient formation of an AT-III, thrombin, and heparin ternary complex. Heparin also mediates the release of tissue factor pathway inhibitor (TFPI) from endothelial cells. TFPI, a heparin cofactor, is a serine protease that directly binds to and inhibits factor X. TFPI is a potent anti-thrombotic, particularly when co-administered with heparin.

In addition to heparin's anticoagulant properties, its complexity and wide distribution in mammals have lead to the suggestion that it may also be involved in a wide range of additional biological activities. Heparin-like glycosaminoglycans, present both at the cell surface and in the extracellular matrix, are a group of complex polysaccharides that are variable in length, consisting of a disaccharide repeat unit composed of glucosamine and an uronic acid (either iduronic or glucuronic acid). The high degree of complexity for HLGAGs arises not only from their polydispersity and the possibility of two different uronic acid components, but also from differential modification at four positions of the disaccharide unit. Three positions, viz., C2 of the uronic acid and the C3, C6 positions of the glucosamine can be O-sulfated. In addition, C2 of the glucosamine can be N-acetylated or N-sulfated. Together, these modifications could theoretically lead to 32 possible disaccharide units, making HLGAGs potentially more information dense than either DNA (4 bases) or proteins (20 amino acids). This enormity of possible structural variants allows HLGAGs to be involved in a large number of diverse biological processes, including angiogenesis (Sasisekharan, R., Moses, M. A., Nugent, M. A., Cooney, C. L. & Langer, R. (1994) Proc Natl Acad Sci USA 91, 1524-8, embryogenesis (Binari, R. C., Staveley, B. E., Johnson, W. A., Godavarti, R., Sasisekharan, R. & Manoukian, A. S. (1997) Development 124, 2623-32; Tsuda, M., Kamimura, K., Nakato, H., Archer, M., Staatz, W., Fox, B., Humphrey, M., Olson, S., Futch, T., Kaluza, V., Siegfried, E., Stam, L. & Selleck, S. B. (1999) Nature 400, 276-80.; and Lin, X., Buff, E. M., Perrimon, N. & Michelson, A. M. (1999) Development 126, 3715-23) and the formation of β-fibrils in Alzheimer's disease (McLaurin, J., Franklin, T., Zhang, X., Deng, J. & Fraser, P. E. (1999) Eur J Biochem 266, 1101-10. And Lindahl, B., Westling, C., Gimenez-Gallego, G., Lindahl, U. & Salmivirta, M. (1999) J Biol Chem 274, 30631-5).

Although heparin is highly efficacious in a variety of clinical situations and has the potential to be used in many others, the side effects associated with heparin therapy are many and varied. Anti-coagulation has been the primary clinical application for unfractionated heparin (UFH) for over 65 years. Due to its erratic pharmacokinetics following s.c. administration, UFH has been administered by intravenous injection instead. Additionally, the application of UFH as an anticoagulant has been hampered by the many side effects associated with non-specific plasma protein binding with UFH.

Side effects such as heparin-induced thrombocytopenia (HIT) are primarily associated with the long chain of UFH, which provides binding domains for various proteins. HIT is an immune-mediated thrombocytopenia which is the result of antibodies, usually IgG, directed against heparin-platelet factor 4 (PF4) complexes. Injected heparin binds with normally occurring low levels of PF4 in plasma to form a macromolecular complex that binds to the surface of platelets. In some patients, antibodies are produced against the heparin/PF4 complex. When present, these antibodies bind to the heparin/

PF4 complex on the surface of platelets and crosslink Fc receptors on the platelet surface thereby causing platelet activation. Platelet activation releases procoagulants including additional PF4. Release of the latter in the presence of heparin further increases platelet activation. The activated platelets either join in forming a clot or are removed by the spleen. Platelet activation ceases when heparin is removed, however, the antibody usually remains detectable for four to six weeks.

Clinically, patients with HIT typically present with a decrease in platelet count, generally five to eleven days after initiated of heparin therapy. Platelet counts drop by up to 50%, to levels usually between 20 and 150 (×103/mm3). This thrombocytopenia is associated with thrombosis rather than purpura or bleeding; deep vein thromboses and pulmonary emboli are the most common complication. Arterial thrombosis occurs less often and usually involves large limb vessels, cerebral arteries, and visceral arteries. It has been estimated that 20% of patients receiving heparin therapy develop heparin induced platelet antibodies, 3% have a drop in platelet count, and 1% or less experience thrombotic complications. Other reported manifestations of heparin-induced thrombocytopenia include localized skin lesions with subcutaneous heparin administration, acute systemic reactions resembling febrile transfusion reactions, and transient global amnesia.

Other side effects include intracranial hemorrhage, bleeding, internal/external hemorrhage, hepatic enzyme (AST and ALT) level elevation, and derma lesion at the site of injection. This has led to the explosion in the generation and utilisation of low molecular weight heparin (LMWH) as an efficacious alternative to UFH. Although attention has been focused on LMWH as heparin substitutes due to their more predictable pharmacological action, reduced side effects, sustained antithrombotic activity, and better bioavailability, there is at present no means of correlating their activity with a particular structure or structural motif due to the structural heterogeneity of heparin and LMWH, as it has been technically unfeasible to determine their structures, and there has been no reliable and readily available means for monitoring LMWH levels in a subject. And since all of the commercially available LMWH preparations are not fully neutralized by protamine, an unexpected reaction could have extremely adverse effects; the anti-Xa activity of enoxaparin and other LMWH are neutralizable only to an extent of about 40% with ≦2 mg Protamine/100 IU anti-Xa LMWH. The anti-IIa activity is neutralizable only to an extent of about 60% with ≦2 mg Protamine/100 IU anti-Xa LMWH. (On the other hand, the anti-Xa and anti-IIa activity of UFH is neutralizable almost completely (>90%) with ≦2 mg Protamine sulfate/100 IU anti-Xa UFH.)

Pharmaceutical preparations of these polysaccharides, typically isolated from porcine intestinal mucosa, are heterogeneous in length and composition. As such, only a portion of a typical preparation possesses anticoagulant activity. At best, the majority of the polysaccharide chains in a pharmaceutical preparation of heparin or LMWH are inactive, at worst, these chains interact nonspecifically with plasma proteins to elicit the side effects associated with heparin therapy. Therefore, it is important to develop novel LMWHs that retain the anticoagulant activity and other desired activities of UFH but have reduced side effects. LMWHs, essentially due to their reduced chains sizes and dispersity, display markedly less non-specific plasma protein binding. However, all LMWHs that are currently clinically available also possess reduced anti-IIa activity as compared to UFH. Because of this decreased activity, a larger dose of LMWH is required (compared to UFH) in order to achieve a similar anti-coagulant activity, and the standard tests for UFH activity, activated partial thromboplastin time (aPTT) or thrombin clotting times (TCT), are not useful as they rely primarily on anti-IIa activity for a readout. The most widely used test for monitoring LMWH levels is an anti-Xa activity test, which depends on the subject having sufficient levels of antithrombin III (ATIII), which is not always the case. This test is quite costly (well over $100.00) and is not routine or readily available, as samples generally must be sent to an outside lab for analysis. Consequently, the use of LMWHs so far has been largely limited to the prevention of thrombosis and not to their treatment, and the population of patients to whom it can be administered has been limited, excluding, among others, pediatric patients, patients with abnormal renal function as measured by RFI, urea, creatinine, phosphorus, glomerular filtration rate (GFR), or BUN (Blood Urea Nitrogen level) in blood and urine and the interventional cardiology patient population. Improved monitoring methods are necessary to provide the advantages of LMWHs to a wider population of patients without increasing the risk of undesired effects. In addition, improved monitoring could allow for courses of therapy tailored to the patients condition throughout the course of their illness, for instance drug preparations given to the patient before a clot has been formed could differ from drug preparations given to the patient shortly after a clot has formed or a longer period of time after a clot has formed.

Although to a lesser degree than UFH, LMWHs are polydisperse and microheterogeneous, with undefined structure, and thus possess inherent variability. Current methods of LMWH preparation lack standardization and result in preparations that may vary substantially from batch to batch in composition and in efficacy.

In an attempt to characterize the molecular, structural, and activity variations of heparin, several techniques have been investigated for the analysis of heparin preparations. Gradient polyacrylamide gel electrophoresis (PAGE) and strong ion exchange HPLC (SAX) have been used for the qualitative and quantitative analysis of heparin preparations. Although the gradient PAGE method can be useful in determining molecular weight, it suffers from a lack of resolution, particularly the lack of resolution of different oligosaccharides having identical size. SAX-HPLC, which relies on detection by ultraviolet absorbance, is often insufficiently sensitive for detecting small amounts of structurally important heparin-derived oligosaccharides. As current technologies for analyzing heparins and other glycosaminoglycans are insufficient, it has been heretofore impossible to create LMWH preparations with any degree of batch-batch consistency, or to predict the potency of a given batch.

SUMMARY OF THE INVENTION

The invention is based in part on the discovery of methods for analyzing heterogeneous populations of sulfated polysaccharides, e.g., heparin, e.g., UFH, LMWH, and synthetic heparins, and methods of producing sulfated polysaccharides having desired properties, e.g., desired activities and/or reduced undesired properties, e.g., undesired side effects. Thus, the invention relates to methods and products associated with analyzing and monitoring heterogeneous populations of sulfated polysaccharides, e.g., to novel methods of analyzing and thus defining the structural signature and activity of heterogeneous populations of sulfated polysaccharides. Therapeutic heparin products including low molecular weight heparin products and methods of producing, analyzing and monitoring these products are described.

In one aspect, the invention provides a method of analyzing a sample, e.g., a composition which includes a polysaccha-

ride. In one embodiment, the composition further comprises one or more tags, antibodies, lectins, or proteins.

A "polysaccharide" as used herein is a polymer composed of monosaccharides linked to one another. In many polysaccharides, the basic building block of the polysaccharide is actually a disaccharide unit, which can be repeating or non-repeating. Thus, a unit when used with respect to a polysaccharide refers to a basic building block of a polysaccharide and can include a monomeric building block (monosaccharide) or a dimeric building block (disaccharide). Polysaccharides include but are not limited to heparin-like glycosaminoglycans, chondroitin sulfate, hyaluronic acid and derivatives or analogs thereof, chitin in derivatives and analogs thereof, e.g., 6-O-sulfated carboxymethyl chitin, immunogenic polysaccharides isolated from phellinus linteus, PI-88 (a mixture of highly sulfated oligosaccharide derived from the sulfation of phosphomannum which is purified from the high molecular weight core produced by fermentation of the yeast pichia holstii) and its derivatives and analogs, polysaccharide antigens for vaccines, and calcium spirulan (Ca-SP, isolated from blue-green algae, spirulina platensis) and derivatives and analogs thereof.

A polysaccharide according to the invention can be a mixed population of polysaccharides, e.g., a heparin, synthetic heparin, or LMWH preparation. As used herein, a "mixed population of polysaccharides" is a polydisperse mixture of polysaccharides. The term "polydisperse" or "polydispersity" refers to the weight average molecular weight of a composition (Mw) divided by the number average molecular weight (Mn). The polydispersity of unfractionated heparin and various LMWHs are known, as are methods for determining polydispersity. Compositions with polydispersity near 1 are more homogeneous, containing fewer different polysaccharides. As an example, a preparation of unfractionated heparin, which contains a wide variety of polysaccharides of differing lengths and compositions, has a polydispersity of about 1.5 to 2.0.

In some embodiments, the sample is derived from a human or veterinary subject, an experimental animal, a cell, or any commercially available preparation of polysaccharides, e.g., UFH or LMWH, including but not limited to enoxaparin (Lovenox™); dalteparin (Fragmin™); certoparin (Sandobarin™); ardeparin (Normiflo™); nadroparin (Fraxiparin™); parnaparin (Fluxum™); reviparin (Clivarin™); tinzaparin (Innohep™ or Logiparin™), or fondaparinux (Arixtra™). In some embodiments, the human or veterinary subject is having, at risk for having, or recovering from a surgical intervention, for example, angioplasty, stent placement, cardiopulmonary bypass procedure, tissue or organ transplant, coronary revascularization surgery, orthopedic surgery, treatment for a fracture such as a hip fracture, hip replacement, knee replacement, PCI, and prosthesis replacement surgery. In some embodiments, the human or veterinary subject is a patient with abnormal renal function as measured by RFI, urea, creatinine, phosphorus, GFR or BUN levels in blood or GFR or urine. In some embodiments, the human or veterinary subject has or is at risk for having complications associated with receiving heparin or LMWH, e.g., HIT. the human or veterinary subject is overweight or obese, for example a subject who is 20, 30, 40, 50 or more pounds overweight. In some embodiments, the human or veterinary subject is extremely thin or frail, for example a subject who is 20, 30, 40, 50 or more pounds underweight, or who is suffering from an immune deficiency, e.g., HIV/AIDS. In some embodiments, the human or veterinary subject is a pediatric patient. In some embodiments, the human or veterinary subject is pregnant. In some embodiments, the human or veterinary subject is a patient having a spinal or epidural hematoma. In some embodiments, the human or veterinary subject is a patient with a prosthetic heart valve. In some embodiments, the human or veterinary subject has an ATIII deficiency or abnormality. In some embodiments, the human or veterinary subject has a factor Xa deficiency or abnormality.

In some embodiments, the method further comprises monitoring for presence, tissue distribution, spatial distribution, temporal distribution or retention time, in a cell or a subject, e.g., an experimental animal. In some embodiments, the method includes determining the structural signature of one or more batches of a product. In some embodiments, the method further includes selecting a batch as a result of the determination. In some embodiments, the method further includes comparing the results of the determination to preselected values, e.g., a reference standard.

In a preferred embodiment, the composition is digested, e.g., chemically and/or enzymatically digested, e.g., incompletely or completely digested. The enzymatic digestion is carried out with a heparin degrading enzyme, e.g., heparinase I, heparinase II, heparinase III, heparinase IV, heparanase or functionally active variants and fragments thereof. The chemical digestion is carried out with a chemical agent, e.g., oxidative depolymerization, e.g., with H_2O_2 or Cu^+ and H_2O_2, deaminative cleavage, e.g., with isoamyl nitrite or nitrous acid, β-eliminative cleavage, e.g., with benzyl ester, and/or by alkaline treatment.

In some embodiments, the sample includes a population of polysaccharides wherein less than or equal to 20% are <2000 Da species, greater than or equal to 68% are 2000-8000 Da species, and less than or equal to 18% are >8000 Da species, or the same as is found in commercially available enoxaparin preparations, preferably with an average molecular weight of about 4500 Da. In some embodiments, the sample has approximately 100 IU/mg anti-Xa activity. In some embodiments, the sample has a pH of 5.5-7.5. In some embodiments, one or more components of the sample is tagged or labeled.

Although the compositions are described in terms of mole %, it is well understood in the art that the compositions may also be described in terms of AUC (area under the curve) or AUC % within the scope of the invention. In some embodiments the composition chemically and/or enzymatically digested, incompletely or completely. The enzymatic digestion is carried out with a heparin degrading enzyme, e.g., heparinase I, heparinase II, heparinase III, heparinase IV, heparanase or functionally active variants and fragments thereof. The chemical digestion is carried out with a chemical agent, e.g., oxidative depolymerization, e.g., with H_2O_2 or Cu^+ and H_2O_2, deaminative cleavage, e.g., with isoamyl nitrite, or nitrous acid, β-eliminative cleavage, e.g., with benzyl ester, and/or by alkaline treatment. In one embodiment, the composition is a HLGAG, and analyzing the composition includes determining the presence of one or more components by optionally fractionating the HLGAG, chemically or enzymatically digesting the HLGAG, and determining the molecular weight of the digested HLGAG.

The method includes analyzing a sample comprising a polysaccharide by providing a structural signature for the polysaccharide. A structural signature, as used herein, refers to information regarding, e.g., the identity and number the mono- and di-saccharide building blocks of a polysaccharide, information regarding the physiochemical properties such as the overall charge (also referred to as the "net charge" or "total charge"), charge density, molecular size, charge to mass ratio and the presence of iduronic and/or glucuronic acid content as well as the relationships between the mono- and di-saccharide building blocks, and active sites associated

with these building blocks, inter alia. The structural signature can be provided by determining one or more primary outputs chosen from the following:

- the presence or the amount of one or more component saccharides or disaccharides; as used herein, "component saccharides" refers to the saccharides that make up the polysaccharide. Component saccharides can include monosaccharides, disaccharides, trisaccharides, etc., and can also include sugars normally found in nature as well as non-natural and modified sugars as defined below, inter alia;
- the presence or the amount of one or more block components, wherein a "block component" is made up of more than one saccharide or polysaccharide;
- the presence or amount of one or more saccharide-representatives, wherein a "saccharide-representative" is a saccharide modified to enhance detectability, including saccharides modified by methods such as chemical modification, enzymatic or chemical digestion, inter alia;
- the presence or amount of an indicator of three dimensional structure or a parameter related to three dimensional structure, e.g., activity, e.g., a structural motif or binding site, e.g., the presence or amount of a structure produced by cross-linking a polysaccharide, e.g., the cross-linking of specific saccharides which are not adjacent in the linear sequence; or
- the presence or amount of one or more modified saccharides, wherein a modified saccharide is one present in a starting material used to make a preparation but which is altered in the production of the preparation, e.g., a saccharide modified by cleavage.

In a preferred embodiment, one can further analyze the polysaccharide by the use of a secondary output, which includes one or more of: total charge; charge/mass ratio, density of charge; sequence; positioning of one or more active site; and polydispersity. "Total charge" of a polysaccharide such as heparin can be calculated by dividing the mass by the average molecular weight of a disaccharide (500) and multiplying that number by the average charge per disaccharide (2.3); or by calculating the charge based on one or more primary outputs, e.g., the identity and number of mono- and di-saccharide building blocks present. "Charge/mass ratio" can be calculated by dividing the total charge by the mass of the polysaccharide. "Density of charge" can be calculated by dividing the total charge by the average length of the polysaccharide. "Sequence" refers to the linear arrangement of covalently linked component saccharides, and can be determined by methods known in the art, e.g., the methods disclosed herein and in WO 00/65521, WO 02/23190, Venkataraman (1999); Shriver et al. (2000a); Shriver et al. (2000b); and Keiser et al. (2001); the entire teachings of which are incorporated herein by reference. "Positioning of the active site" refers to a correlation between a certain component polysaccharide and a given activity. In a preferred embodiment, the structural signature is determined by one or more methods chosen from the group consisting of MALDI-MS, ESI-MS, CE, HPLC, FPLC, fluorometry, ELISA, chromogenic assays, colorimetric assays, NMR and other spectroscopic techniques.

Some of the methods and compositions described herein are described with the use of one of the primary outputs, e.g., the amount of one or more component saccharides or disaccharides. However, it is to be understood that any of the above mentioned outputs can be used with, or in place of the output actually recited in the methods and compositions described herein.

In another aspect, the invention features a method of analyzing a polysaccharide drug, e.g., a heparin, synthetic heparin, or LMWH. The method includes:

- providing or determining a first structural signature, e.g., any structural signature described herein for a batch of drug having a first level of preselected patient reaction, e.g., a preselected level of negative or positive reaction to the drug;
- providing or determining a second structural signature, e.g., any structural signature described herein, for a second batch of drug having a second level of preselected patient reaction, e.g., a preselected level of negative or positive reaction to the drug;
- comparing the first and second structural determination to associate a property of the drug, e.g., a chemical or structural property, with a preselected level of patient reaction. For example, one can determine the structure of a batch of drug having a relatively high level of unwanted effects, determine the structure of a batch of drug having a relatively low level of unwanted effects, and then compare the structural determinations of the two batches to correlate a property of the drug with the unwanted effects. In some embodiments, the method further includes selecting or discarding a batch of drug having a property correlated with the high or the low level of patient reaction.

As used herein, "batch" refers to a quantity of anything produced at one operation, e.g., a quantity of a compound produced all at one operation. A "batch of drug" is a quantity of a drug that was produced at one operation, e.g., in a single process.

The invention relates in part to novel methods of analyzing and thus defining the structural signature and activity of heterogeneous populations of sulfated polysaccharides. The invention provides methods to correlate structure with function (referred to as Compositional Analysis Method (CAM)) to identify key structural motifs, easily measured, that can be used to predict the activity of and monitor the levels of a heparin. The methods of the invention can be utilized to create glycoprofiles to standardize polysaecharide preparations such as heparin, synthetic heparin, and low molecular weight heparins with increased activity and bioavailability in vivo while maintaining a desired degree of consistency from batch to batch. The invention provides new, reliable and consistent preparations of polysaecharides, particularly of LMWHs, that have enhanced properties as compared to the current generation of commercially available LMWHs, as well as methods for preparing such preparations.

In one aspect, the invention is a method of analyzing the structural signature of a sample, e.g., a composition as described herein, including detecting the presence of a number of components, e.g., $I/GH_{NAc,6S}I/GH_{NS,3S,6S}$, $I/GH_{NS,6S}GH_{NS,3S,6S}$, $I/GH_{NAc,6S}GH_{NS,3S}$, $I/GH_{NS,6S}I/GH_{NS,3S}$, $I/GH_{NS,6S}I/GH_{NS,3S,6S}$, $I/GH_{NAc,6S}GH_{NS,3S}$, $I/GH_{NS,6S}I/GH_{NS,3S}$ or combinations thereof, as well as non-natural, e.g., modified, sugars. These signatures can be detected as is (e.g., by measuring their molecular weight, and sequencing, or by NMR, etc.) or can be detected indirectly by detecting their derivatives, e.g., $\Delta UH_{NAc,6S}GH_{NS,3S,6S}$, $\Delta UH_{NS,6S}GH_{NS,3S,6S}$, $\Delta UH_{NAc,6S}GH_{NS,3S}$, $\Delta UH_{NS,6S}GH_{NS,3S}$, $\Delta UH_{NS,6S}GH_{NS,3S,6S}$, $\Delta UH_{NAc,6S}GH_{NS,3S}$, $\Delta UH_{NS,6S}GH_{NS,3S}$ or combinations thereof, as well as non-natural, e.g., modified, sugars. As used herein, "non-natural sugars" refers to sugars having a structure that does not normally exist in heparin in nature. As used herein, "modified sugars" refers to sugars derived from natural sugars, which have a structure that does not normally exist in a polysaccha-

ride in nature, which can occur in a LMWH as a result of the methods used to make the LMWH, such as the purification procedure. The results of this method are a set of values representing the glycoprofile of the composition.

As used herein, "p1" or "peak 1" refers to $\Delta U_{2S}H_{NS,6S}$; "p2" or "peak 2" refers to $\Delta U_{2S}H_{NS}$; "p3" or "peak 3" refers to $\Delta UH_{NS,6S}$; "p4" or "peak 4" refers to $\Delta U_{2S}H_{NAC,6S}$; "p5" or "peak 5" refers to ΔUH_{NS}; "p6" or "peak 6" refers to $\Delta U_{2S}H_{NAC}$; "p7" or "peak 7" refers to $\Delta UH_{NAC,6S}$; "p8" or "peak 8" refers to $\Delta UH_{NAc,6S}GH_{NS,3S,6S}$; $\Delta UH_{NS,6S}GH_{NS,3S,6S}$; $\Delta UH_{NAc,6S}GH_{NS,3S}$; or $\Delta UH_{NS,6S}GH_{NS,3S}$, collectively. "p9" or "peak 9" and "p10" or "peak 10" refer to the non-natural sugars associated with peaks 9 and 10, respectively. The nomenclature "ΔU" refers to an unsaturated uronic acid (iduronic acid (I) or glucuronic acid (G) that has a double bond introduced at the 4-5 position as a result of the lyase action of heparinases. Upon the introduction of the double bond the distinction between the stereo isomers I and U disappears, and hence the notation ΔU: Δ to denote double bond, and U to denote that they can be derived from either I or G. Thus, as used herein, "ΔU" represents both I and G, such that $\Delta U_{2S}H_{NS,6s}$ encompasses both $I_{2S}H_{NS,6S}$ and $G_{2S}H_{NS,6S}$; $\Delta U_{2S}H_{NS}$ encompasses both $I_{2S}H_{NS}$ and $G_{2S}H_{NS}$, and so forth. While the compositions of the invention are described as mole % of different building blocks, it is well known in the art that they can also be described as AUC %, as weight %, or by other known terminology within the scope of the invention.

A further embodiment of the invention relates to the use of a method described herein for analyzing a sample, e.g., a composition including a mixed population of polysaccharides, such as glycosaminoglycans (GAGs), HLGAGS, UFH, FH, or LMWHS. This method includes, inter alia, providing the composition; and determining if one or more, e.g., two, three, four, five six, or seven, of the following are present in a preselected range: $I/G_{2S}H_{NS,6S}$ (e.g., 15-85 mole %); $I/G_{2S}H_{NS}$ (e.g., 0.1-20 mole %); $I/G\ H_{NS,6S}$ (e.g., 0.1-20 mole %); $I/G_{2S}H_{NAc,6S}$ (e.g., 0.1-10 mole %); $I/G\ H_{NS}$ (e.g., 0.1-10 mole %); $I/G_{2S}H_{Nac}$ (e.g., 0.1-5 mole %); $I/GH_{NAc,6S}GH_{NS,3S,6S}$; $I/GH_{NS,6S}GH_{NS,3S,6S}$; $I/GH_{NAc,6S}GH_{NS,3S}$; or $I/GH_{NS,6S}GH_{NS,3S}$ or a mixture thereof (e.g., 0.1-20 mole %); by measuring their representative building blocks, e.g., $\Delta U_{2S}H_{NS,6S}$; $\Delta U_{2S}H_{NS}$; $\Delta UH_{NS,6S}$; $\Delta U_{2S}H_{NAc,6S}$; ΔUH_{NS}; $I/G_{2S}H_{NAc}$; $\Delta UH_{NAc,6S}$; $\Delta UH_{NAc,6S}GH_{NS,3S,6S}$; $\Delta UH_{NS,6S}GH_{NS,3S,6S}$; $\Delta UH_{NAc,6S}GH_{NS,3S}$; or $\Delta UH_{NS,6S}GH_{NS,3S}$; thereby analyzing the composition. In some embodiments, the method includes determining if all of the foregoing are present in a preselected range. As used herein, "in a preselected range" also includes and is satisfied by all lesser included ranges.

In some embodiments, the method includes determining if $\Delta U_{2S}H_{NS,6S}$ is present in the range of 45-80 mole %, 50-75 mole %, 55-70 mole %, or 60-65 mole %.

In some embodiments, the method includes determining if $\Delta U_{2S}H_{NS}$ is present in the range of 2-15 mole %, 5-10 mole %, or 6-9 mole %.

In some embodiments, the method includes determining if $\Delta UH_{NS,6S}$ is present in the range of 5-18 mole %, 7-15 mole %, or 10-12 mole %.

In some embodiments, the method includes determining if $\Delta U_{2S}H_{NAc,6S}$ is present in the range of 0.5-7.5 mole %, 1-5 mole % or 1.5-3 mole %.

In some embodiments, the method includes determining if ΔUH_{NS} is present in the range of 1-7 mole %, 2-5 mole % or 3-4 mole %.

In some embodiments, the method includes determining if $\Delta U_{2S}H_{NAc}$ is present in the range of 0.1-5 mole %, 0.5-3 mole % or 1-2.5 mole %.

In some embodiments, the method includes determining if $\Delta UH_{Nac,6S}$ is present in the range of 0.1-12 mole %, 0.5-10 mole % or 1-6 mole %.

In some embodiments, the method includes determining if $\Delta UH_{NAc,6S}GH_{NS,3S,6S}$; $\Delta UH_{NS,6S}GH_{NS,3S,6S}$; $\Delta UH_{NAc,6S}GH_{NS,3S}$; $\Delta UH_{NS,6S}GH_{NS,3S}$ or a mixture thereof is present in the range of 1-15 mole %; 2-10 mole %; 3-8 mole %; or 5-7 mole %.

In another embodiment, this method includes determining whether non-natural sugars are present in sample, e.g., a composition as described herein, in a preselected range, generally 0.1-5 mole %; 0.1-2.5 mole %; 0.1-1 mole %. In some embodiments, the method includes determining whether the non-natural sugar of peak 9 is present in the range of 0.1-5 mole %, 0.1-2.5 mole %, or 0.1-1 mole %. In some embodiments, the method includes determining whether the non-natural sugar of peak 10 is present in the range of 0.1-5 mole %, 0.1-2.5 mole %, or 0.1-1 mole %. In some embodiments, the method includes determining whether peak 11 is present in the range of 0.1-10 mole %, 1-5 mole %, or 2-4 mole %.

Thus, in another aspect, the invention includes a method of analyzing a sample by providing the sample and determining if a non-natural sugar, e.g., a modified sugar, is present in the sample. The non-natural sugar can be peak 9, peak 10, and/or peak 11.

In some embodiments, the method further includes detecting one or more biological activities of the sample, such as an effect on cellular activities such as undesired cell growth or proliferation; cellular migration, adhesion, or activation; neovascularization; angiogenesis; coagulation; HIT propensity; and inflammatory processes. In some embodiments the biological activity is anti-Xa activity; anti-IIa activity; FGF binding; protamine neutralization; and/or PF4 binding.

In some embodiments, the method can also include correlating one or more biological activities to the structural signature of the sample. In some embodiments, the method can also include creating a reference standard having information correlating the biological activity to the structural signature. This reference standard can be used, e.g., to predict the level of activity of a sample, e.g., a LMWH preparation. Thus, in another aspect, the invention provides a method for predicting the level of activity of a LMWH preparation by determining the structural signature of the LMWH preparation and comparing the determined structural signature to the reference standard described herein. The activity can be an effect on cellular activities such as cell growth or proliferation; cellular migration, adhesion, or activation; neovascularization; angiogenesis; coagulation; and inflammatory processes. In some embodiments, the activity is anti-Xa activity, anti-IIa activity, FGF binding, protamine neutralization, and/or PF4 binding.

In another aspect, the invention also provides a method of analyzing a sample of a heparin having a selected biological activity by determining if a component known to be correlated with the selected activity is present in the sample. The method can further include determining the level of the component, e.g., the mole % or AUC % of the component. The activity can be an effect on cellular activities such as cell growth or proliferation; cellular migration, adhesion, or activation; neovascularization; angiogenesis; coagulation; and inflammatory processes, anti-Xa activity, anti-IIa activity, FGF binding, protamine neutralization, and/or PF4 binding. In some embodiments, the presence of $U_{2S}H_{NS}$, $U_{2S}H_{Nac,6S}$, $U_{2S}H_{Na}$, and/or $U_{2S}H_{NS,6S}$, e.g., in a range of 0.1-100 mole %, is indicative of PF4 binding activity. In some embodiments, the presence of $\Delta UH_{NAc,6S}GH_{NS,3S,6S}$; $\Delta UH_{NS,6S}GH_{NS,3S,6S}$;

$\Delta UH_{NAc,6S}GH_{NS,3S}$; $\Delta UH_{NS,6S}GH_{NS,3S}$ or a mixture thereof, e.g., in the range of 0.1-100 mole %, is indicative of anti-Xa activity.

In a preferred embodiment, the method further includes analyzing a plurality of compositions to determine the structural signature of each composition; detecting the biological activity of each composition; comparing the structural signature of the compositions to the detected biological activities; and correlating the biological activity with a structural signature or component thereof, e.g., a primary or secondary output of said structural signature. As used herein, "plurality" means two or more. The biological activity can be, e.g., effects on cellular activities such as undesired cell growth or proliferation; cell death (necrotic or apoptotic); cellular migration, adhesion, or activation; neovascularization; angiogenesis; coagulation; and inflammatory processes. In a preferred embodiment, the biological activity can include one or more of anti-Xa activity, anti-IIa activity, FGF binding, protamine neutralization, TFPI release, and/or PF4 binding.

In some embodiments, the biological activity-structural correlation information can be used to design a heparin, synthetic heparin, or LMWH preparation for a specific indication, e.g., renal impairment, autoimmunity, disease associated with coagulation, such as thrombosis, cardiovascular disease, vascular conditions or atrial fibrillation; migraine, atherosclerosis; an inflammatory disorder, such as autoimmune disease or atopic disorders; an allergy; a respiratory disorder, such as asthma, emphysema, adult respiratory distress syndrome (ARDS), cystic fibrosis, or lung reperfusion injury; a cancer or metastatic disorder; an angiogenic disorder, such as neovascular disorders of the eye, osteoporosis, psoriasis, and arthritis, Alzheimer's, or is undergoing or having undergone surgical procedure, organ transplant, orthopedic surgery, treatment for a fracture such as a hip fracture, hip replacement, knee replacement, percutaneous coronary intervention (PCI), stent placement, angioplasty, coronary artery bypass graft surgery (CABG). The specific indication can include cellular activities such as cell growth or proliferation; neovascularization; angiogenesis; cellular migration, adhesion, or activation; and inflammatory processes.

In another aspect the invention relates to a method of making one or more batches of a polysaccharide preparation, wherein one or more of the glycoprofile values of the batches varies less than a preselected range. In another aspect, the invention relates to a composition comprising multiple batches of a polysaccharide preparation, wherein one or more of the glycoprofile values for each batch varies less than a preselected range from a pre-selected desired glycoprofile. In some embodiments, the method includes determining the structural signature of one or more batches of a product, and selecting a batch as a result of the determination. In some embodiments, the method can also include comparing the results of the determination to preselected values, e.g., a reference standard. In other embodiments, the method can further include adjusting the dose of the batch to be administered, e.g., based on the result of the determination of the structural signature. Thus, in another aspect the invention relates to a method of determining a reference standard for a composition, e.g., a drug, by analyzing a sample, e.g., a sample including a composition including a mixed population of polysaccharides, such as glycosaminoglycans (GAGs), HLGAGs, UFH, FH, or LMWHs, including but not limited to enoxaparin (Lovenox™); dalteparin (Fragmin™); certoparin (Sandobarin™); ardeparin (Normiflo™); nadroparin (Fraxiparin™); parnaparin (Fluxum™); reviparin (Clivarin™); tinzaparin (Innohep™ or Logiparin™), or Fondaparinux (Arixtra™), and determining if one or more of the following are present in a preselected range: $\Delta U_{2S}H_{NS,6S}$; $\Delta U_{2S}H_{NS}$; $\Delta UH_{NS,6S}$; $\Delta U_{2S}H_{NAc,6S}$; ΔUH_{NS}; $\Delta U_{2S}H_{NAc}$; $\Delta UH_{NAc,6S}$; $\Delta UH_{NAc,6S}GH_{NS,3S,6S}$; $\Delta UH_{NS,6S}GH_{NS,3S,6S}$; $\Delta UH_{NAc,6S}GH_{NS,3S}$; or $\Delta UH_{NS,6S}GH_{NS,3S}$; and/or I/$GH_{NAc,6S}GH_{NS,3S,6S}$; I/$GH_{NS,6S}GH_{NS,3S,6S}$; I/$GH_{NAc,6S}GH_{NS,3S}$; or IG $H_{NS,6S}GH_{NS,3S}$ or a mixture thereof; thereby determining a reference standard for the composition. In some embodiments, the method includes determining if all of the foregoing are present in a preselected range, e.g., peak 1, $\Delta U_{2S}H_{NS,6S}$ (e.g., 15-85 mole %); peak 2, $\Delta U_{2S}H_{NS}$ (e.g., 0.1-20 mole %); peak 4, $\Delta U_{2S}H_{NAC,6S}$ (0.1-10 mole %); peak 6, $\Delta U_{2S}H_{NAC}$ (0.1-5 mole %); and/or peak 8, I/$GH_{NAc,6S}GH_{NS,3S,6S}$; I/$GH_{NS,6S}GH_{NS,3S,6S}$; I/$GH_{NAc,6S}GH_{NS,3S}$ or I/$GH_{NS,6S}GH_{NS,3S}$ or a mixture thereof (e.g., 0.1-20 mole %). In one embodiment, the dose or amount to be administered to a patient is adjusted depending on the level of peak 8 present; e.g., to maintain the levels of anti-Xa/IIa activity, e.g., to maintain a dose of 100 IU of anti-Xa activity.

In one embodiment, the invention relates to a method of determining a reference standard for a drug by analyzing the composition and determining the bioequivalence and/or bioavailability of one or more of the components in the mixture. As used herein, "bioequivalence" means "the absence of a significant difference in the rate and extent to which an active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions."

As used herein, "bioavailability" is "the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action." For compounds that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by a measurement intended to reflect the rate and/or extent to which the active ingredient or active moiety becomes available at the site of action. From a pharmacokinetic perspective, bioavailability data for a given formulation provide an estimate of the relative fraction of the orally administered dose that is absorbed into the systemic circulation when compared to the bioavailability data for a solution, suspension, subcutaneous or intravenous dosage form. Bioavailability studies may provide other pharmacokinetic information related to distribution, elimination, the effects of nutrients on absorption of the drug, dose proportionality, and/or linearity in pharmacokinetics of the active moieties and, where appropriate, inactive moieties. Bioavailability data may also provide information indirectly about the properties of a drug substance prior to entry into the systemic circulation, such as permeability and the influence of presystemic enzymes and/or transporters (e.g., p-glycoprotein). Bioavailability for orally administered drug products may be documented by developing a systemic exposure profile obtained from measuring the concentration of active ingredients and/or active moieties and, when appropriate, its active metabolites over time in samples collected from the systemic circulation.

Several in vivo and in vitro methods can be used to measure product quality bioavailability and establish bioequivalence. These include pharmacokinetic, pharmacodynamic, clinical, and in vitro studies.

As used herein, "pharmacokinetic" refers to the kinetics of release of the drug substance from the drug product into the systemic circulation, as well as clearance, volume of distribution, and absorption, as determined by physiological variables (e.g. gastric emptying, motility, pH). Pharmacokinetics may be evaluated in an accessible biological matrix such as blood, plasma, and/or serum. Pharmacokinetic measure-

ments may also include AUC, does-dependency of activity, peak levels in plasma, time to peak, disposition half-life, and terminal half-life.

As used herein, "pharmacodynamic" refers to defining factors that cause variability in clinical drug response using general assessments, including bone densitometry and caliper total body fat; pulmonary assessments, including pulmonary function testing, expired nitric oxide, pulmonary imaging; Cardiovascular assessments, including cardiac monitoring, ambulatory blood pressure; Holter monitoring, telemetry, ECG, vital signs, cardiac imaging; Nervous system assessments, including electroencephalography, mental function testing, psychomotor function testing, pharmacokinetic EEG; ENT assessments, including audiometric testing, acoustic rhinometry, intraocular pressure, digital retinography; and gastrointestinal assessments, including gastric pH monitoring, endoscopy, imaging, and/or gastric motility.

Thus in one aspect, the invention relates to a method for determining bioequivalence. The method includes some or all of the following: providing or determining the structural signature of a first composition; providing or determining the bioavailability of the first composition; providing or determining the structural signature of a second composition; providing or determining the bioavailability of the second composition; and comparing the structural compositions and bioavailability of the first and second compositions. In some embodiments, bioavailability is determined determining the absorbance characteristics of the composition in one or more subjects, e.g., human or veterinary subjects or experimental animals; and determining the clearance characteristics of the composition in one or more subjects, e.g., human or veterinary subjects or experimental animals.

The invention also includes methods for monitoring subjects receiving polysaccharides. Until now, subjects receiving heparins and HLGAG preparations have been monitored by testing their activated partial thromboplastin time (aPTT) or thrombin clotting times (TCT). However, this test depends in large part on the activity and availability of other substances endogenous to the subject such as fibrinogen and factor VIII, and thus may not give an accurate indication of actual levels. Furthermore, this test is also dependent on the presence of significant anti-IIa activity, which is substantially absent in the LMWHs currently known in the art. Patients receiving heparin but demonstrating an inadequate aPTT response can be evaluated using an anti-Xa assay. A quantitative anti-Xa assay is necessary for monitoring heparin in patients with a prolonged aPTT that may be related to lupus anticoagulants or deficiencies of factor XII and the contact factors (prekallikrein and high molecular weight kininogen); current anti-Xa assays are expensive, take a long time, and are not readily available, so a need exists for a new method of following anti-Xa levels.

Thus the invention also relates to methods of monitoring a subject receiving a polysaccharide, comprising monitoring the level of one or more of the components of the polysaccharide being administered. In one embodiment, the invention relates to monitoring the levels of a single component. In a further embodiment, the invention relates to monitoring the level of a component associated with a biological activity of the polysaccharide. In another embodiment, the invention relates to monitoring a subject receiving a polysaccharide comprising monitoring the levels of components of the polysaccharide correlating to anti-IIa activity or to anti-Xa activity. In other aspect, the methods can include monitoring a subject receiving a polysaccharide, e.g., a LMWH, by monitoring the levels, e.g., serum levels, of one or more components of the polysaccharide correlating to an activity, e.g., PF4 binding. The methods of the invention include monitoring hexasaccharide and octasaccharide fractions of heparins in plasma without prior heparinase digestion; smaller fragments may be monitored following treatment of the sample with an agent as described herein, such as a heparinase or a chemical digestive agent.

Thus in another aspect the invention provides a method of analyzing a sample or a subject, e.g., a sample from a subject, for a heparin having anti-Xa activity. In some embodiments, the sample comprises a bodily fluid, e.g., blood or a blood-derived fluid, or urine. In some embodiments, the heparin comprises UFH or a LMWH, e.g., a LMWH having anti-Xa activity, M118, M115, M411, M108, M405, M312, enoxaparin; dalteparin; certoparin; ardeparin; nadroparin; pamaparin; reviparin; tinzaparin, or fondaparinux. The method can include some or all of the following: providing a sample, e.g., from a subject, e.g., a human or veterinary subject or an experimental animal; determining if one or more components chosen from the group consisting of $\Delta UH_{NAc,6S}GH_{NS,3S,6S}$; $\Delta UH_{NS,6S}GH_{NS,3S,6S}$; $\Delta UH_{NAc,6S}GH_{NS,3S}$; $\Delta UH_{NS,6S}GH_{NS,3S}$ or a fragment or fragments thereof is present in the sample; and optionally, measuring the level of the component or components. In some embodiments, the steps are repeated, e.g., at pre-selected intervals of time, e.g., every two to twenty-four hours, every four to twelve hours, every six to ten hours, continuous monitoring. In some embodiments, the method can also include establishing a baseline, e.g., a baseline for the component or components prior to the subject receiving the heparin. In some embodiments, the method also includes determining if $\Delta U_{2S}H_{NS,6S}$; $\Delta U_{2S}H_{NS}$; $\Delta UH_{NS,6S}$; $\Delta U_{2S}H_{NAC,6S}$; ΔUH_{NS}; $\Delta U_{2S}H_{NAC}$; or $\Delta UH_{NAC,6S}$ is present in the sample. In some embodiments, the method further comprises determining if the components of one or more of peak 9, peak 10, or peak 11 is present in the sample. In some embodiments, the method also includes monitoring for presence, tissue distribution, spatial distribution, temporal distribution or retention time, in a cell or a subject, e.g., an experimental animal. In some embodiments, the method also includes determining the structural signature of one or more batches of a product. In some embodiments, the method also includes selecting a batch as a result of the determination. In some embodiments, the method also includes comparing the results of the determination to preselected values, e.g., a reference standard.

In some embodiments, the determination step includes purifying the sample; optionally fractionating the sample; contacting the sample with at least one agent and determining the structural signature of the digested sample. The agent can be an enzyme, e.g., a heparin degrading enzyme, e.g., heparinase I, heparinase II, heparinase III, heparinase IV, heparanase and functionally active variants and fragments thereof, or a chemical agent, e.g., H_2O_2, Cu^+ and H_2O_2, isoamyl nitrite, nitrous acid, benzyl ester or alkaline treatment.

In some embodiments, the determination step includes: optionally purifying the sample, contacting the sample with a reagent specific for one or more of the components, e.g., a peptide, protein, lectin, or antibody; and detecting the binding of the antibody to the component. In some embodiments, the determination includes determining if one or more components chosen from the group consisting of $\Delta UH_{NAc,6S}GH_{NS,3S,6S}$; $\Delta UH_{NS,6S}GH_{NS,3S,6S}$; $\Delta UH_{NAc,6S}GH_{NS,3S}$; $\Delta UH_{NS,6S}GH_{NS,3S}$ or a fragment or fragments thereof is present in the range of 0.1-20 mole %.

In some embodiments, the human or veterinary subject is having, at risk for having, or recovering from a surgical intervention, for example, angioplasty, stent placement, cardiopulmonary bypass procedure, tissue or organ transplant, coro-

nary revascularization surgery, orthopedic surgery, treatment for a fracture such as a hip fracture, hip replacement, knee replacement, PCI, and prosthesis replacement surgery. In some embodiments, the human or veterinary subject is a patient with abnormal renal function as measured by RFI, urea, creatinine, phosphorus, GFR or BUN levels in blood or GFR or urine. In some embodiments, the human or veterinary subject has or is at risk for having complications associated with receiving heparin or LMWH, e.g., HIT. the human or veterinary subject is overweight or obese, for example a subject who is 20, 30, 40, 50 or more pounds overweight. In some embodiments, the human or veterinary subject is extremely thin or frail, for example a subject who is 20, 30, 40, 50 or more pounds underweight, or who is suffering from an immune deficiency, e.g., HIV/AIDS. In some embodiments, the human or veterinary subject is a pediatric patient. In some embodiments, the human or veterinary subject is pregnant. In some embodiments, the human or veterinary subject is a patient having a spinal or epidural hematoma. In some embodiments, the human or veterinary subject is a patient with a prosthetic heart valve. In some embodiments, the human or veterinary subject has an ATIII deficiency or abnormality. In some embodiments, the human or veterinary subject has a factor Xa deficiency or abnormality.

In some embodiments, the method further comprises monitoring for presence, tissue distribution, spatial distribution, temporal distribution or retention time, in a cell or a subject, e.g., an experimental animal. In some embodiments, the method includes determining the structural signature of one or more batches of a product. In some embodiments, the method further includes selecting a batch as a result of the determination. In some embodiments, the method further includes comparing the results of the determination to preselected values, e.g., a reference standard.

In some embodiments, the sample includes a population of polysaccharides wherein less than or equal to 20% are <2000 Da species, greater than or equal to 68% are 2000-8000 Da species, and less than or equal to 18% are >8000 Da species, or the same as is found in commercially available enoxaparin preparations, preferably with an average molecular weight of about 4500 Da. In some embodiments, the sample has approximately 100 IU/mg anti-Xa activity. In some embodiments, the sample has a pH of 5.5-7.5. In some embodiments, one or more components of the sample is tagged or labeled.

In another aspect, the invention provides a method of analyzing a sample or a subject, e.g., monitoring a subject receiving a heparin having anti-IIa activity. In some embodiments, the sample comprises a bodily fluid, e.g., blood or a blood-derived fluid, or urine. In some embodiments, the heparin comprises UFH or a LMWH, e.g., a LMWH having anti-Xa activity, M118, M115, M411, M108, M405, M312, enoxaparin; dalteparin; certoparin; ardeparin; nadroparin; parnaparin; reviparin; tinzaparin, or fondaparinux. The method includes some or all, typically all, of the following: providing a sample, e.g,. from a subject, e.g., a human or veterinary subject, or an experimental animal; and determining if one or more structural signature outputs known to be associated with anti-IIa activity is present in the sample; and optionally, determining the level of the component or components. In some embodiments, one or more of the steps are repeated at preselected intervals of time, e.g., every two to twenty-four hours, every four to twelve hours, every six to ten hours, or continuously.

In some embodiments, the structural signature output associated with anti-IIa activity is a polysaccharide comprising at least one of $\Delta UH_{NAc,6S}GH_{NS,3S,6S}$, $\Delta UH_{NS,6S}GH_{NS,3S,6S}$, $\Delta UH_{NAc,6S}GH_{NS,3S}$, or $\Delta UH_{NS,6S}GH_{NS,3S}$ with one or more other disaccharide units. In some embodiments, the method further comprises establishing a baseline for the component or components prior to the subject receiving the heparin. In some embodiments, the method further comprises monitoring presence, tissue distribution, spatial distribution, temporal distribution or retention time, in a cell or a subject, e.g., an experimental animal In some embodiments, the method includes determining the structural signature of one or more batches of a product. In some embodiments, the method further includes selecting a batch as a result of the determination. In some embodiments, the method further includes comparing the results of the determination to preselected values, e.g., a reference standard.

In some embodiments, the determination step includes purifying the sample; optionally fractionating the sample; contacting the sample with at least one agent; and determining the structural signature of the digested sample. The agent can be an enzyme, e.g., a heparm degrading enzyme, e.g., heparinase I, heparinase II, heparinase III, heparinase IV, heparanase and functionally active variants and fragments thereof, or a chemical agent, e.g., H_2O_2, Cu^+ and H_2O_2, isoamyl nitrite, nitrous acid, benzyl ester or alkaline treatment.

In some embodiments, the determination step includes: optionally purifying the sample, contacting the sample with a reagent specific for one or more of the components, e.g., a peptide, protein, lectin, or antibody; and detecting the binding of the antibody to the component. In some embodiments, the determination includes determining if one or more components chosen from the group consisting of $\Delta UH_{NAc,6S}GH_{NS,3S,6S}$; $\Delta UH_{NS,6S}GH_{NS,3S,6S}$; $\Delta UH_{NAc,6S}GH_{NS,3S}$; $\Delta UH_{NS,6S}GH_{NS,3S}$ or a fragment or fragments thereof is present in the range of 0.1-20 mole %.

In some embodiments, the human or veterinary subject is having, at risk for having, or recovering from a surgical intervention, for example, angioplasty, stent placement, cardiopulmonary bypass procedure, tissue or organ transplant, coronary revascularization surgery, orthopedic surgery, treatment for a fracture such as a hip fracture, hip replacement, knee replacement, PCI, and prosthesis replacement surgery. In some embodiments, the human or veterinary subject is a patient with abnormal renal function as measured by RFI, urea, creatinine, phosphorus, GFR or BUN levels in blood or GFR or urine. In some embodiments, the human or veterinary subject has or is at risk for having complications associated with receiving heparin or LMWH, e.g., HIT. the human or veterinary subject is overweight or obese, for example a subject who is 20, 30, 40, 50 or more pounds overweight. In some embodiments, the human or veterinary subject is extremely thin or frail, for example a subject who is 20, 30, 40, 50 or more pounds underweight, or who is suffering from an immune deficiency, e.g., HIV/AIDS. In some embodiments, the human or veterinary subject is a pediatric patient. In some embodiments, the human or veterinary subject is pregnant. In some embodiments, the human or veterinary subject is a patient having a spinal or epidural hematoma. In some embodiments, the human or veterinary subject is a patient with a prosthetic heart valve. In some embodiments, the human or veterinary subject has an ATIII deficiency or abnormality. In some embodiments, the human or veterinary subject has a factor Xa deficiency or abnormality.

In some embodiments, the method further comprises monitoring for presence, tissue distribution, spatial distribution, temporal distribution or retention time, in a cell or a subject, e.g., an experimental animal. In some embodiments, the method includes determining the structural signature of one or more batches of a product. In some embodiments, the

method further includes selecting a batch as a result of the determination. In some embodiments, the method further includes comparing the results of the determination to preselected values, e.g., a reference standard.

In some embodiments, the sample includes a population of polysaccharides wherein less than or equal to 20% are <2000 Da species, greater than or equal to 68% are 2000-8000 Da species, and less than or equal to 18% are >8000 Da species, or the same as is found in commercially available enoxaparin preparations, preferably with an average molecular weight of about 4500 Da. In some embodiments, the sample has approximately 100 IU/mg anti-Xa activity. In some embodiments, the sample has a pH of 5.5-7.5. In some embodiments, one or more components of the sample is tagged or labeled.

In another aspect, the invention provides a method of analyzing a sample or a subject, e.g., monitoring a LMWH in sample or a subject. The method includes some or all, typically all, of the following: providing a sample, e.g., from a subject, e.g., a human or veterinary subject, or an experimental animal; and determining if one or more non-natural sugars, e.g., modified sugars, are present in the sample; and optionally, determining the level of the non-natural sugar. In some embodiments, the LMWH is enoxaparin. In some embodiments, the non-natural sugars are benzylated. In some embodiments, the non-natural sugars comprise one or more of peaks 9 and 10. In some embodiments, the sample comprises a bodily fluid, e.g., blood or a blood-derived bodily fluid, or urine. In some embodiments, one or more of the steps are repeated at pre-selected intervals of time, e.g., every two to twenty-four hours, every four to twelve hours, every six to ten hours, continuously.

In some embodiments, the determination step includes purifying the sample; optionally fractionating the sample; contacting the sample with at least one agent and determining the structural signature of the digested sample. The agent can be an enzyme, e.g., a heparin degrading enzyme, e.g., heparinase I, heparinase II, heparinase III, heparinase IV, heparanase and functionally active variants and fragments thereof, or a chemical agent, e.g., H_2O_2, Cu^+ and H_2O_2, isoamyl nitrite, nitrous acid, benzyl ester or alkaline treatment.

In some embodiments, the determination step includes: optionally purifying the sample, contacting the sample with a reagent specific for one or more of the components, e.g., a peptide, protein, lectin, or antibody; and detecting the binding of the antibody to the component. In some embodiments, the determination includes determining if one or more components chosen from the group consisting of $\Delta UH_{NAc,6S}GH_{NS,3S,6S}$; $\Delta UH_{NS,6S}GH_{NS,3S,6S}$; $\Delta UH_{NAc,6S}GH_{NS,3S}$; $\Delta UH_{NS,6S}GH_{NS,3S}$ or a fragment or fragments thereof is present in the range of 0.1-20 mole %.

In some embodiments, the human or veterinary subject is having, at risk for having, or recovering from a surgical intervention, for example, angioplasty, stent placement, cardiopulmonary bypass procedure, tissue or organ transplant, coronary revascularization surgery, orthopedic surgery, treatment for a fracture such as a hip fracture, hip replacement, knee replacement, PCI, and prosthesis replacement surgery. In some embodiments, the human or veterinary subject is a patient with abnormal renal function as measured by RFI, urea, creatinine, phosphorus, GFR or BUN levels in blood or GFR or urine. In some embodiments, the human or veterinary subject has or is at risk for having complications associated with receiving heparin or LMWH, e.g., HIT. the human or veterinary subject is overweight or obese, for example a subject who is 20, 30, 40, 50 or more pounds overweight. In some embodiments, the human or veterinary subject is extremely thin or frail, for example a subject who is 20, 30, 40, 50 or more pounds underweight, or who is suffering from an immune deficiency, e.g., HIV/AIDS. In some embodiments, the human or veterinary subject is a pediatric patient. In some embodiments, the human or veterinary subject is pregnant. In some embodiments, the human or veterinary subject is a patient having a spinal or epidural hematoma. In some embodiments, the human or veterinary subject is a patient with a prosthetic heart valve. In some embodiments, the human or veterinary subject has an ATIII deficiency or abnormality. In some embodiments, the human or veterinary subject has a factor Xa deficiency or abnormality.

In some embodiments, the method further comprises monitoring for presence, tissue distribution, spatial distribution, temporal distribution or retention time, in a cell or a subject, e.g., an experimental animal. In some embodiments, the method includes determining the structural signature of one or more batches of a product. In some embodiments, the method further includes selecting a batch as a result of the determination. In some embodiments, the method further includes comparing the results of the determination to preselected values, e.g., a reference standard.

In some embodiments, the sample includes a population of polysaccharides wherein less than or equal to 20% are <2000 Da species, greater than or equal to 68% are 2000-8000 Da species, and less than or equal to 18% are >8000 Da species, or the same as is found in commercially available enoxaparin preparations, preferably with an average molecular weight of about 4500 Da. In some embodiments, the sample has approximately 100 IU/mg anti-Xa activity. In some embodiments, the sample has a pH of 5.5-7.5. In some embodiments, one or more components of the sample is tagged or labeled.

In another aspect, the invention relates to a method of analyzing a polysaccharide drug, e.g., a heparin, synthetic heparin, or LMWH comprising the steps of:

a. determining a first structural signature, e.g., any structural signature described herein for a first batch of drug having a first level of preselected patient reaction, e.g., a preselected level of negative or positive reaction to the drug;

b. determining a second structural signature, e.g., any structural signature described herein, for a second batch of drug having a second level of preselected patient reaction, e.g., a preselected level of negative or positive reaction to the drug; and

c. comparing the first and second structural signature determinations to determine the presence or absence of a correlation between a property of the drug, e.g., a chemical or structural property, with a preselected level of patient reaction.

As used herein, "preselected patient reaction" refers to any reaction of interest, whether it be a positive or negative reaction. For instance, a positive patient reaction might be anticoagulation, shrinkage of a tumor, surgical intervention without occurrence of complications such as thrombosis, e.g., deep vein thrombosis; non-occurrence of ischemic complications of unstable angina and/or non-Q-wave myocardial infarction; relief of deep vein thrombosis; and non-occurrence of thromboembolic complications due to severely restricted mobility during acute illness. A negative patient reaction might be epidural or spinal hematoma; hemorrhage; thrombocytopenia; elevations of serum aminotransferases; local irritation, pain, hematoma, ecchymosis, and erythema; anemia; ecchymosis; fever; nausea; edema; peripheral edema; dyspnea; confusion; diarrhea; pneumonia; atrial fibrillation; Heart failure; Lung edema; local reactions at the injection site (i.e., skin necrosis, nodules, inflammation, oozing); systemic allergic reactions (i.e., pruritus, urticaria, anaphylactoid reac-

tions); vesiculobullous rash; purpura; thrombocytosis; thrombocytopenia with thrombosis; hyperlipidemia; hyperlipidemia with marked hypertriglyceridemia; ataxia, decreased motility; cyanosis; and coma.

In another aspect, the invention relates to selecting a safer, less variable LMWH to use for treating a patient, by determining the structure structural signature of a first batch of drug having a relatively high level of undesirable patient reactions, determining the structural signature of a second batch of drug having a relatively low level of undesirable patient reactions, and selecting a primary or secondary output correlated with the high or the low level of patient reactions. As used herein, "desirable patient reaction" refers to, inter alia, a preselected positive patient reaction as defined above. As used herein, "undesirable patient reaction" refers to an unwanted patient reaction, such as a negative patient reaction as defined above.

The invention also relates to the development of a "bed side" monitoring system based upon assaying a more purified form of an active component of LMWH using assay techniques known in the art including, but not limited to chromogenic reagents and ELISA techniques.

In another aspect, the invention relates to a method of treating patients that have been excluded from LMWH treatment such as obese patients, pediatric patients, patients with abnormal renal function as measured by RFI, urea, creatinine, phosphorus, GFR or BUN in blood and urine and the interventional cardiology patient population by monitoring a subject receiving a polysaccharide, comprising monitoring the level of one or more of the components of the polysaccharide being administered. In another aspect, the invention relates to a method of treating patients with complications of LMWH by monitoring a subject receiving a polysaccharide, comprising monitoring the level of one or more of the components of the polysaccharide being administered. In another aspect, the invention relates to the selection of a LMWH for treatment of a patient previously excluded from LMWH treatment because of an elevated risk of a negative patient reaction, by selecting a LMWH that has a low level or none of a primary or secondary output associated with a negative patient reaction. The invention further relates to LMWH compositions comprising one or more non-natural sugar components. In a preferred embodiment, the non-natural sugar component may be the sugars associated with peaks **9**, **10** and **11**. In a related aspect, the invention relates to methods of monitoring a subject receiving a LMWH having a non-natural sugar component, the method comprising monitoring the subject for levels of the non-natural sugar, preferably in the bodily fluid of the subject.

The invention also relates to a method of determining the safety of compositions including a mixed population of polysaccharides, such as glycosaminoglycans (GAGs), HLGAGs, UFH, FH, or LMWHs including but not limited to enoxaparin (Lovenox™); dalteparin (Fragmin™); certoparin (Sandobarin™); ardeparin (Normiflo™); nadroparin (Fraxiparin™); parnaparin (Fluxum™); reviparin (Clivarin™); tinzaparin (Innohep™ or Logiparin™) e, or Fondaparinux (Arixtra™) in the treatment of subtypes of renal disease.

The invention also relates to a method for further understanding the mechanism of action of a specific heparin, LMWH or synthetic heparin and differentiating it from other heparins, LMWHs or synthetic heparins by analyzing and defining the structural signature and activity of one or more of the heparins, LMWHs or synthetic heparins in a heterogeneous population of sulfated polysaccharides.

The invention also relates to a method for further understanding the mechanism of action of specific, individual components of specific heparins, LMWHs or synthetic heparins and thereby differentiating it from other heparins, LMWHs or synthetic heparins by analyzing and defining the structural signature and activity of specific components. The invention further relates to a method for specifically identifying components of heparins, LMWHs or synthetic heparins which bind to proteins or other molecules which are associated with disease states or negative patient reactions, using, inter alia, chip-based specific affinity assays such as those disclosed in Keiser, et al., *Nat Med* 7, 123-8 (2001). This chip-based approach to assess the binding of heparin fragments to various proteins may be readily used to assay an array of plasma and other proteins and assess binding properties.

The invention also relates to a method for broadening the therapeutic utility of heparins, LMWHs or synthetic heparins for use in areas other than as modulators of hemostasis, by understanding the mechanism of action of specific, individual components of specific heparins, LMWHs or synthetic heparins by analyzing and defining the structural signature and activity of specific components and the effect those components can have in the treatment of a specific disease.

The invention also relates to a method for broadening the therapeutic utility of heparins, LMWHs or synthetic heparins for use in areas other than as modulators of hemostasis, by designing compositions with enhanced activities for these diseases by analyzing and defining the structural signature and activity of specific components and the effect those components can have in the treatment of a specific disease. In one embodiment, the method can include selecting a heparin, LMWH or synthetic heparin, based, e.g., on the presence or absence of a structural signature associated with a specific activity, e.g., a preselected range of structural signatures associated with a specific activity. In one embodiment, the method can include designing a composition of a heparin, LMWH, or synthetic heparin for treatment of subjects determined to be at risk for thrombocytopenia, e.g., heparin-induced thrombocytopenia (HIT), for example, patients having an immunodeficiency, e.g., AIDS, HIV, hereditary immunodeficiency or immunodeficiency caused by an environmental or medicinal agents (e.g., a chemotherapeutic agent or radiation treatment.) The heparin, LMWH or synthetic heparin composition can be designed to treat such subjects, e.g., a composition that has decreased PF4 binding, e.g., has a decreased amount of one or more of the following, e.g., one or more of the following is decreased as compared to a preselected range: peak 1, $\Delta U_{2S}H_{NS,6S}$ (e.g., less than about 60 mole %, e.g., 15-30 mole %); peak 2, $\Delta U_{2S}H_{NS}$ (e.g., less than about 5 mole %, e.g., 1.8-3.5 mole %); peak 4, $\Delta U_{2S}H_{NAC,6S}$ (e.g., less than about 2 mole %, e.g., 0.1-1.0 mole %); and/or peak 6, $\Delta U_{2S}H_{NAC}$ (e.g., less than about 2 mole %, e.g., 0.1-0.5 mole %). Preferably, the composition has decreased amounts of all of the following: peak 1: $\Delta U_{2S}H_{NS,6S}$; peak 2: $\Delta U_{2S}H_{NS}$; peak 4: $\Delta U_{2S}H_{NAC,6S}$; and/or peak 6: $\Delta U_{2S}H_{NAC}$.

The invention also relates to broadening the therapeutic utility of heparins, LMWHs or synthetic heparins for treating clot bound thrombin by designing novel LMWHs of smaller sizes, and/or of increased anti-IIa activity that are active and can reach and treat the thrombus.

The invention also relates to a method for designing heparins, LMWHs or synthetic heparins with ideal product profiles including, but not limited to such features as high activity, having both anti-Xa and anti-IIa activity, titratable, well characterized, neutralizable, lower side effects including reduced HIT, attractive pharmacokinetics, and/or reduced PF4 binding that allow for optional monitoring and can be practically manufactured by analyzing and defining the structural signature and activity of specific components of a com-

position that includes a mixed population of polysaccharides, such as glycosaminoglycans (GAGs), HLGAGs, UFH, FH, LMWHs, or synthetic heparins including but not limited to enoxaparin (Lovenox™); dalteparin (Fragmin™); certoparin (Sandobarin™); ardeparin (Normiflo™); nadroparin (Fraxiparin™); parnaparin (Fluxum™); reviparin (Clivarin™); tinzaparin (Innohep™ or Logiparin™), or Fondaparinux (Arixtra™) and enriching for components with desired activities and de-enriching for components with undesirable activities. As used herein, "desired activities" refers to those activities that are beneficial for a given indication, e.g., a positive patient reaction as defined herein, inter alia. An "undesirable activity" may include those activities that are not beneficial for a given indication, e.g., a negative patient reaction, as defined herein, inter alia. A given activity may be a desired activity for one indication, and an undesired activity for another, such as anti-IIa activity, which while undesirable for certain indications, is desirable in others, notably acute or trauma situations, as discussed above.

The invention also relates to a method for designing novel heparins, LMWHs or synthetic heparins with different or ideal anti-IIa activities using rational design based upon knowing that anti-Xa activity requires at least a pentasaccharide with a critical 3-O sulfate group on an internal glucosamine, anti-IIa activity requires longer saccharides and the positional orientation between the pentasaccharide and the thrombin binding site is crucial. The method can also include designing novel heparins, LMWHs or synthetic heparins using rational design based upon knowing that decreased PF4 binding requires the reduced presence of peaks 1, 2, 4 and 6, e.g., the presence of these peaks is reduced as compared to UFH, e.g., the presence of these peaks at less than about 60 mole % of peak 1, e.g., 15-30 mole %; less than about 5 mole % of peak 2, e.g., 1.5-3.5 mole %; less than about 2 mole % of peak 4, e.g., 0.1-1.5 mole % and/or less than about 2 mole % of peak 6, e.g., 0.1-0.5 mole %.

The invention also relates to novel heparins made by the methods of the invention, e.g., novel heparins, LMWHs or synthetic heparins with desired product profiles including, but not limited to such features as high activity, both anti-Xa and anti-IIa activity, titratability, well characterized, neutralizable (e.g. by protamine), reduced side effects including reduced HIT, and/or attractive pharmacokinetics, that allow for optional monitoring, and novel heparins, LMWHs or synthetic heparins with different or enhanced anti-IIa activities. Thus in one aspect, the invention includes a LMWH preparation having an increased or decreased ratio of anti-IIa activity and anti-Xa activity, e.g., a LMWH preparation made by the methods described herein.

In another aspect, the invention includes a panel of two or more LMWH preparations having different ratios of anti-IIa activity and anti-Xa activity, e.g., LMWH preparations made by the methods described herein.

In one aspect, the method includes a method of producing a LMWH preparation having or not having a pre-selected biological activity. The method can include some or all of the following: providing one or more aliquots of heparin; optionally fractionating the heparin; modifying the aliquots of heparin under conditions designed to produce the activity; and optionally purifying the digested aliquots. In some embodiments, the desired biological activity is an effect on cellular activities such as cell growth or proliferation; cellular migration, adhesion, or activation; neovascularization; angiogenesis; coagulation; and inflammatory processes. In some embodiments, the desired biological activity is anti-IIa activity; anti-Xa activity; platelet factor 4 binding; FGF binding; or sensitivity to neutralization with protamine. In some embodiments, the desired biological activity is anti-IIa activity and anti-Xa activity. In some embodiments, the aliquots are modified by chemically or enzymatically digesting the FH or UFH, e.g., by enzymatic digestion carried out using one or more heparin degrading enzymes, e.g., heparinase I, heparinase II, heparinase III, heparinase IV, heparanase or functionally active variants and fragments thereof. In some embodiments, the chemical digestion is carried out by a chemical chosen from the group consisting of oxidative depolymerization with H_2O_2 or Cu^+ and H_2O_2, deaminative cleavage with isoamyl nitrite, or nitrous acid, β-eliminative cleavage with benzyl ester or by alkaline treatment. In some embodiments, the method also includes testing the LMWH preparation for the desired biological activity.

In another aspect, the invention also includes a LMWH preparation prepared by the methods described herein, e.g., a LMWH preparation having anti-IIa activity and anti-Xa activity.

In another aspect, the invention provides a LMWH composition having both anti-Xa and anti-IIa activity comprising less than or equal to 20% <2000 Da species, greater than or equal to 68% 2000-8000 Da species, and less than or equal to 18% >8000 Da species, preferably with an average molecular weight of about 4500 Da, wherein the anti-Xa activity is >50% neutralizable by protamine and the anti-IIa activity is >70% neutralizable by protamine. In some embodiments, the LMWH composition has approximately 100 IU/mg anti-Xa activity. In some embodiments, the LMWH composition has a pH of 5.5-7.5. In some embodiments, the LMWH composition comprises $\Delta U_{2S}H_{NS,6S}$ in the range of 15-85 mole %; $\Delta U_{2S}H_{NS}$ in the range of 0.1-20 mole %; $\Delta UH_{NS,6S}$ in the range of 0.1-20 mole %; $\Delta U_{2S}H_{NAc,6S}$ in the range of 0.1-10 mole %; ΔUH_{NS} in the range of 0.1-10 mole %; $\Delta U_{2S}H_{NAc}$ in the range of 0.1-5 mole %; $\Delta UH_{NAc,6S}$ in the range of 0.1-15 mole %; and $\Delta UH_{NAc,6S}GH_{NS,3S,6S}$ in the range of 0.1-20 mole %. In some embodiments, the LMWH composition is free of or substantially free of non-natural sugars. In some embodiments, the LMWH composition further comprises greater than 30 IU/mg anti-IIa activity.

In another aspect, the invention provides a LMWH that is substantially free of non-natural sugars, e.g., the sugars associated with peaks 9 and 10, and comprising less than or equal to 20% <2000 Da species, greater than or equal to 68% 2000-8000 Da species, and less than or equal to 18% >8000 Da species, preferably with an average molecular weight of about 4500 Da. In some embodiments, the LMWH composition has approximately 100 IU/mg anti-Xa activity. In some embodiments, the LMWH composition has a pH of 5.5-7.5. In some embodiments, the LMWH composition comprises $\Delta U_{2S}H_{NS,6S}$ in the range of 15-85 mole %; $\Delta U_{2S}H_{NS}$ in the range of 0.1-20 mole %; $\Delta UH_{NS,6S}$ in the range of 0.1-20 mole %; $\Delta U_{2S}H_{NAc,6S}$ in the range of 0.1-10 mole %; ΔUH_{NS} in the range of 0.1-10 mole %; $\Delta U_{2S}H_{NAc}$ in the range of 0.1-5 mole %; $\Delta UH_{NAc,6S}$ in the range of 0.1-15 mole %; and $\Delta UH_{NAc,6S}GH_{NS,3S,6S}$ in the range of 0.1-20 mole %. In some embodiments, the LMWH composition further comprises greater than 30 IU/mg anti-IIa activity.

In another aspect, the invention provides a LMWH which, as compared with enoxaparin, is enriched, e.g., has 5%, 10%, or 20% more non-natural sugars, e.g., the sugars associated with peaks 9, 10, 11, or 12, than enoxaparin, and comprising less than or equal to 20% <2000 Da species, greater than or equal to 68% 2000-8000 Da species, and less than or equal to 18% >8000 Da species, preferably with an average molecular weight of about 4500 Da. In some embodiments, the LMWH composition has approximately 100 IU/mg anti-Xa activity. In some embodiments, the LMWH composition has a pH of

5.5-7.5. In some embodiments, the LMWH composition comprises $\Delta U_{2S}H_{NS,6S}$ in the range of 15-85 mole %; $\Delta U_{2S}H_{NS}$ in the range of 0.1-20 mole %; $\Delta UH_{NS,6S}$ in the range of 0.1-20 mole %; $\Delta U_{2S}H_{NAc,6S}$ in the range of 0.1-10 mole %; ΔUH_{NS} in the range of 0.1-10 mole %; $\Delta U_{2S}H_{NAc}$ in the range of 0.1-5 mole %; $\Delta UH_{NAc,6S}$ in the range of 0.1-15 mole %; and $\Delta UH_{NAc,6S}GH_{NS,3S,6S}$ in the range of 0.1-20 mole %. In some embodiments, the LMWH composition further comprises greater than 30 IU/mg anti-IIa activity.

In other aspects, the invention relates to a composition including a mixed population of polysaccharides, such as glycosaminoglycans (GAGs), HLGAGs, UFH, FH, LMWHs or synthetic heparins including but not limited to enoxaparin (Lovenox™); dalteparin (Fragmin™); certoparin (Sandobarin™); ardeparin (Normiflo™); nadroparin (Fraxiparin™); parnaparin (Fluxum™); reviparin (Clivarin™); tinzaparin (Innohep™ or Logiparin™) or Fondaparinux (Arixtra™) with less batch-batch variability.

In other aspects, the invention relates to a composition including a mixed population of polysaccharides, such as glycosaminoglycans (GAGs), HLGAGs, UFH, FH, or LMWHs where the anti-Xa activity can be fully neutralized by protamine, e.g., the anti-Xa activity can be neutralized by >50%.

In other aspects, the invention relates to a composition including a mixed population of polysaccharides, such as glycosaminoglycans (GAGs), HLGAGs, UFH, FH, or LMWHs where the anti-IIa activity can be fully neutralized, e.g., the anti-IIa activity can be neutralized by ≧70%.

In other aspects, the invention relates to a composition including a mixed population of polysaccharides, such as glycosaminoglycans (GAGs), HLGAGs, UFH, FH, or LMWHs where the composition has lower PF4 binding sequences.

In other aspects, the invention relates to a composition including a mixed population of polysaccharides, such as glycosaminoglycans (GAGs), HLGAGs, UFH, FH, or LMWHs where the process to make the composition has been optimized to ensure lower PF4 binding sequences. In some embodiments, the composition includes reduced amounts of peak 1, peak 2, peak 4, and/or peak 6 relative to UFH, e.g., peak 1, $\Delta U_{2S}H_{NS,6S}$ (e.g., less than about 50 mole %, e.g., 15-30 mole %); peak 2, $\Delta U_{2S}H_{NS}$ (e.g., less than about 5 mole %, e.g., 1.8-3.5 mole %); peak 4, $\Delta U_{2S}H_{NAC,6S}$ (e.g., less than about 2 mole %, e.g., 0.1-1.0 mole %); and/or peak 6, $\Delta U_{2S}H_{NAC}$ (e.g., less than about 2 mole %, e.g., 0.1-0.5 mole %).

In other aspects, the invention relates to compositions made by the methods of the invention including ultra-low molecular weight heparins (ULMWHs) comprising 15-20 monosaccharide units, optionally with binding affinity (Kd) for ATIII of 1-60 nM, anti-Xa activity of 5-30 nm (IC50) and/or anti-IIa activity of 0.5-100 or greater than 500 nM (IC50). These ULMWHs may also be susceptible to neutralization by protamine and/or substantially free of binding affinity for PF4.

In other aspects, the invention relates to compositions made by the methods of the invention including comprising LMWHs with mean molecular weight from 1500-3000 D, anti-Xa activity in the range of 94-150 IU/mg, preferably 125-150 IU/mg, more preferably 140-150 IU/mg; anti-IIa of ≦10 IU/mg, preferably ≦5 IU/mg; and an anti-Xa:anti-IIa activity ratio greater than 10:1, preferably greater than 25:1, optionally including at least one sulfated polysaccharide of heparin having 2-26 saccharide units.

In other aspects, the invention relates to a LMWH composition comprising a tag. In some embodiments, the tag emits detectable electromagnetic radiation. In some embodiments, the tag is a fluorescent label, a mass-label compatible with mass-spectrometric methods, O18, yttrium, 3H, affinity label, pH sensitive label, or radioactive label. In another aspect, the invention provides a method of evaluating a sample for the presence of a LMWH comprising a tag comprising the steps of providing a sample; optionally purifying the sample; and determining the presence of the tag in the sample. In some embodiments, the method also includes the step of determining the level of the tag. In some embodiments, the sample is from a subject, e.g., a human or veterinary subject or an experimental animal as described herein, receiving the LMWH comprising a tag. In some embodiments, the LMWH is M118, M115, M411, M108, M405, M312, enoxaparin; dalteparin; certoparin; ardeparin; nadroparin; parnaparin; reviparin; tinzaparin, or fondaparinux. In some embodiments, the sample is a bodily fluid, e.g., blood, blood plasma, and/or urine. In another aspect, the invention includes a kit for performing a method for evaluating a sample for the presence of a LMWH as described herein, including one or more of the following: a tag; a compound for attaching the tag to a polysaccharide, and a standard, e.g., a polysaccharide or a tagged polysaccharide.

The invention also relates to LMWH compositions comprising a marker or tag; in a preferred embodiment, the invention relates to LMWHs comprising a marker or tag that can be detected using an ELISA or chromogenic assay. In a preferred embodiment, the marker or tag may be an antibody, fluorescent label, a mass-label compatible with mass-spectrometric methods, an affinity label, a radioactive label, UV label, NMR label, ESR or EPR spin label, or other chromophore. In a further preferred embodiment, the marker or tag may be attached to a component of the LMWH having biological activity. In a related aspect, the invention relates to methods of monitoring a subject receiving a LMWH having a marker or tag, the method comprising monitoring the subject for the presence and/or levels of the marker or tag, preferably in the bodily fluid of the subject. The invention further relates to a kit for detecting such a marker or tag.

The compositions of the invention may be derived from a natural source or may be synthetic. In some embodiments, the natural source is porcine intestinal mucosa.

The compositions may be formulated for in vivo delivery in some embodiments. For instance, the preparation may be formulated for inhalation, oral, subcutaneous, intravenous, intraperitoneal, transdermal, buccal, sublingual, parenteral, intramuscular, intranasal, intratracheal, ocular, vaginal, rectal, transdermal, and/or sublingual delivery.

Optionally, the compositions may also include one or more additives. Additives include, but are not limited to, dermatan sulfate, heparan sulfate or chondroitin sulfate.

In some embodiments of the invention, the preparation includes a specific amount of heparin. For instance the preparation may include 80-100 mole % heparin, 60-80 mole % heparin, 40-60 mole % heparin, or 20-40 mole % heparin. The heparin may, for example, be LMWH, native heparin, heparin sulfate, biotechnology-derived heparin, chemically modified heparin, synthetic heparin or heparin analogues.

In other aspects, the invention relates to methods for treating or preventing disease using the compositions of the invention. For instance, the invention includes methods for treating or preventing a condition in a subject wherein the subject has or is at risk of a disorder selected from the group consisting of: disease associated with coagulation, such as thrombosis, cardiovascular disease, vascular conditions or atrial fibrillation; migraine, atherosclerosis; an inflammatory disorder, such as autoimmune disease or atopic disorders; an allergy; a respi-

ratory disorder, such as asthma, emphysema, adult respiratory distress syndrome (ARDS), cystic fibrosis, or lung reperfusion injury; a cancer or metastatic disorder; an angiogenic disorder, such as neovascular disorders of the eye, osteoporosis, psoriasis, and arthritis; Alzheimer's; bone fractures such as hip fractures; or is undergoing or having undergone surgical procedure, organ transplant, orthopedic surgery, hip replacement, knee replacement, percutaneous coronary intervention (PCI), stent placement, angioplasty, coronary artery bypass graft surgery (CABG). The compositions of the invention are administered to a subject having or at risk of developing one or more of the diseases in an effective amount for treating or preventing the disease.

In other aspects, the invention relates to a method for treating or preventing disease using different and specific novel LMWHs with specific product profiles at different phases in the course of treatment of a patient by dosing the patient with a LMWH having an enhanced activity for a specific disease state, e.g., a high level of anti-Xa or -IIa activity and than dosing with another LMWH composition having an enhanced activity for the changed disease state, e.g., having decreased PF4 binding.

In some aspects, the invention provides a method of treating a subject, e.g. a human or veterinary subject. The method includes some or all of the following: providing a panel of two or more LMWH preparations having different ratios of anti-IIa activity and anti-Xa activity; selecting a LMWH preparation having a desired ratio; and administering one or more doses of a therapeutically effective amount of the LMWH preparation to the subject.

In some embodiments, the method also includes monitoring the levels of LMWH in the subject, e.g., repeatedly monitoring the levels of LMWH in the subject over time. In some embodiments, the method includes adjusting the doses of the LMWH preparation. In some embodiments, the method includes monitoring the status of the subject in response to the administration of the LMWH preparation. In some embodiments, the method monitoring the status of the subject over a period of time. In some embodiments, the method also includes administering a different LMWH preparation based on changes in the status of the subject over time. In another aspect, the invention features a method of inhibiting coagulation in a patient by administering one or more doses of a therapeutic amount of a LMWH preparation described herein having high anti-Xa and anti-IIa activity, monitoring the status of the subject, then administering one or more doses of a therapeutic amount of a LMWH preparation as described herein having high anti-Xa activity alone. In some embodiments, the method includes providing or determining the structural signature of the LMWH preparation, and optionally correlating the status of the subject to the structural signature of the LMWH.

In another aspect, the invention provides a method of treating a subject who has previously been diagnosed with HIT, comprising administering to the subject a therapeutically effective dose of a composition described herein having decreased PF4 binding activity.

In another aspect, the invention provides a method for determining the safety or suitability of a heparin for use in a particular indication. The method includes some or all, typically all, of the following: providing the structural signature of the heparin; providing a reference structural signature; determining if the heparin is acceptable, e.g., by comparing the structural signature of the heparin with the reference structural signature; where a preselected index of similarity is met, the heparin is safe or suitable. In some embodiments, the reference structural signature is associated with one or more undesired effects. In some embodiments, the reference structural signature is associated with one or more desired effects. In a preferred embodiment, the safety or suitability of the heparin is determined based on the level of peak 1, 2, 4, and/or 6 present in the sample; e.g., batches with lower levels of one or more of peak 1, 2, 4, and/or 6 are safer than batches with higher levels.

In another aspect, the invention provides a method of making one or more batches of a LMWH preparation which has a batch-to-batch variation of a preselected range from a preselected value for one or more component saccharide chosen from the group consisting of $\Delta U_{2S}H_{NS,6S}$; $\Delta U_{2S}H_{NS}$; $\Delta UH_{NS,6S}$; $\Delta U_{2S}H_{NAC,6S}$; ΔUH_{NS}; $\Delta U_{2S}H_{NAC}$; $\Delta UH_{NAC,6S}$; and $\Delta UH_{NAC,6S}GH_{NS,3S,6S}$. The method includes some or all, typically all of the following: selecting a desired value; providing an aliquot of UFH; optionally fractionating the aliquot; determining the level of the component in the aliquot; and selecting a batch or batches with less than the preselected range of variation from the desired value. In some embodiments, the preselected variation is less than 2.5%, more preferably less than 2% or less than 1%. In some embodiments, the preselected variation for p1 is less than 3%, less than 2%, or less than 1%. In some embodiments, the preselected variation for p2 is less than 16%, less than 15%, less than 1%, less than 10%, less than 5%, less than 1%. In some embodiments, the preselected variation for p3 is less than 8%, less than 4%, less than 2%, less than 1%. In some embodiments, the preselected variation for p4 is less than 22%, less than 15%, less than 10%, less than 5%, less than 1%. In some embodiments, the preselected variation for p5 is less than 3%, less than 2%, or less than 1%. In some embodiments, the preselected variation for p6 is less than 10%, less than 5%, less than 2%, less than 1%. In some embodiments, the preselected variation for p7 is less than 90%, less than 75%, less than 50%, less than 25%, less than 10%, less than 5%. In some embodiments, the preselected variation for p8 is less than 12%, less than 10%, less than 8%, less than 5%, less than 4%, less than 2%.

In another aspect, the invention provides a method of making one or more batches of a LMWH preparation which has a batch-to-batch variation of less than a preselected range from a preselected value, e.g., less than 2.5%, more preferably less than 2% or less than 1%, for one or more component saccharide chosen from the group consisting of p1-p8. The method includes some or all, typically all, of the following: selecting a value; providing an aliquot of UFH or LMWH; precipitating the aliquot; optionally subjecting the aliquot to an ion exchange process; and contacting the aliquot with an agent under conditions such that the desired value will result. In some embodiments, the agent is a heparin degrading enzyme chosen from the group consisting of heparinase I, heparinase II, heparinase III, and functionally active variants and fragments thereof. In some embodiments, the agent is a chemical, e.g., a chemical chosen from the group consisting of H_2O_2, Cu^+ and H_2O_2, isoamyl nitrite, nitrous acid, benzyl ester or alkaline treatment. In another aspect, the invention provides a composition comprising multiple batches of a LMWH preparation prepared by the method described herein. In another aspect, the invention provides a composition comprising a LMWH preparation prepared by a method described herein.

In another aspect, the invention provides a composition comprising multiple batches of a LMWH preparation, wherein, the for each of the batches, the mole % of one or more component chosen from the group consisting of p1-p8 varies less than a preselected variation, e.g., less than 2.5%, more preferably less than 2% or less than 1%.

In another aspect, the invention provides a composition comprising multiple batches of a LMWH preparation,

wherein the glycoprofile of each of the batches for one or more component chosen from the group consisting of p9-p10 varies less than a preselected variation, e.g., less than 2.5%, more preferably less than 2% or less than 1%.

In another aspect, the invention also provides methods for adjusting the dose of a batch of a LMWH to be administered, e.g., depending on the glycoprofile of the LMWH. For example, the dose may be adjusted depending on the level of a peak, e.g., peaks 1, 2, 4, 6, and/or 8. In a preferred embodiment, the dose of the batch is adjusted based on the level of peak 8 present in the batch.

In another aspect, the invention provides a record, e.g., a computer readable record, having an element which identifies a polysaccharide, e.g., UFH or LMWH, an element which identifies one or more components of the polysaccharide, and an element which identifies a range of mole % of the components.

Each of the limitations of the invention can encompass various embodiments of the invention. It is, therefore, anticipated that each of the limitations of the invention involving any one element or combinations of elements can be included in each aspect of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**A. Capillary electrophoresis (CE) profile of enoxaparin (Lovenox™). The different building blocks are labeled as 1, 2, 3 etc., corresponding to the different peaks.

FIG. **1**B. CE spectrum of peak 1 which has been isolated from enoxaparin and re-injected into the CE to ascertain its purity.

FIGS. **2**A and **2**B: Line plots of anti-IIa (**2**A) and anti-Xa (**2**B) values of UFH, UFH size fractionated through Bio-gel P10 column, a LMWH generated as described herein, and commercial LMWHs. There is a linear correlation between the anti-Xa/IIa values, and the mole % peak 8 content of the molecules.

FIG. **3**. Graph of plasma anti-Xa pharmacokinetics of M118, UFH, Enoxaparin and M312 given by s.c. administration in rabbits at 3 mg/kg.

FIG. **4**A Bar graph representing total occlusion time as a function of different heparins (UFH, enoxaparin, M118, and M312) as well as at different doses.

FIG. **4**B. Bar graph representing thrombus weight as a function of heparin treatment at different doses for LFH, enoxaparin, M118, and M312. Thrombus was weighed at the end of the 1 hour thrombus induction period.

FIG. **5**. Line graph of TFPI release profiles after s.c. administration of different heparins at 3 mg/kg. The release of TFPI is reflected by percentage increase in the plasma TFPI activity as determined by a chromogenic assay.

FIG. **6**. Line graph of In vitro protamine neutralization of various LMWH (enoxaparin, M118, and M312) and UFH as a function of their anti-Xa activity is depicted here. M118, M312, and UFH are neutralized by using ≦2 mg/100 IU of heparin/LMWH while enoxaparin has about 60% of its anti-Xa activity still remaining even after using ≧3 mg/100 IU enoxaparin.

FIG. **7**. Line graph of In vitro protamine neutralization of various LMWH (enoxaparin, M118, and M312) and UFH as a function of their anti-IIa activity is depicted here. M118, M312, and UFH are neutralized by using ≦2 mg/100 IU of heparin/LMWH while enoxaparin has about 40% of its anti-IIa activity still remaining even after using ≧3 mg/100 IU enoxaparin.

FIG. **8**. Bar graph of In vivo protamine neutralization of enoxaparin, M118 and M312.

FIG. **9**. Line graph depicting the linear relationship between the amount of PF4 binding components (peaks 1, 2, 4 and 6) in a LMWH preparation and PF4 binding propensity.

FIG. **10**A. CE profile of a LMWH in Blood.

FIG. **10**B. CE profile of the same LMWH in Urine.

FIG. **11**A. CE profile of enoxaparin in plasma before (top panel), at five minutes after administration (middle panel) and at thirty minutes after administration (bottom panel).

FIG. **11**B. CE profile of enoxaparin in urine at different time points upon the administration of enoxaparin, showing the presence of peaks 1, 2, 3, and 4.

FIG. **12**. Line graph showing the clearance of different building blocks of heparin and LMWH (enoxaparin, and other LMWH) in urine was tracked as a function of time. % of building blocks refers to the % of building blocks p1 (peak 1), p8 (peak 8) or p5 (peak 5), as a fraction of the total building blocks seen at that particular time point.

DETAILED DESCRIPTION

The invention involves significant advances in methods of analysis and monitoring of polysaccharides, particularly sulfated polysaccharides such as heparin and LMWHs, and improved compositions for therapeutic treatment. For instance, it has been discovered that the methods described herein can be used to analyze compositions of sulfated GAGs including HLGAGs such as UFH and LMWH, and to create a set of primary and secondary outputs referred to herein as a "structural signature" that indicates, inter alia, the composition and structure of a preparation and can be used to predict the activity of the composition. Further, this information can be used to standardize the production of LMWH compositions, thus resulting in LMWHs with less batch-batch variability and improved ratios of desirable and undesirable activities. For instance, polysaccharides having a high anti-Xa activity are particularly useful for treating coagulation disorders and cardiovascular disease, such as pulmonary embolism, acute myocardial infarction or unstable angina. In addition, polysaccharides having reduced PF4 binding are desirable.

It has also been discovered that polysaccharides having a low anti-Xa activity are particularly useful for treating atherosclerosis, respiratory disorder, a cancer or metastasis, inflammatory disorder, allergy, angiogenic disorder, and/or lung, kidney, heart, gut, brain, or skeletal muscle ischemial-reperfusion injuries. Respiratory disorders include but are not limited to asthma, emphysema, and adult respiratory distress syndrome (ARDS). Angiogenic disorders include but are not limited to neovascular disorders of the eye, osteoporosis, psoriasis, and arthritis. Thus, it is possible to tailor a compounds which would be particularly useful for treating a subject that is preparing to undergo, is undergoing or is recovering from a surgical procedure or is undergoing a tissue or organ transplant. Surgical procedures include but are not limited to cardiac-pulmonary by-pass surgery, coronary revascularization surgery, orthopedic surgery, prosthesis replacement surgery, treatment of fractures including hip fractures, PCI, hip replacement, knee replacement, and stent placement or angioplasty.

It has also been discovered that a polysaccharide having a high anti-IIa activity has beneficial therapeutic properties; for instance, when delivered via a pulmonary delivery system, the rapid onset of action of polysaccharides having high anti-IIa activity is useful in treating acute conditions. Thus the instant invention relates to compositions with high anti-IIa activity for use in treatment of acute cardiac syndrome and myocardial infarction.

It was previously believed in the prior art that a high anti-IIa activity was not desirable for therapeutic purposes. As a result, polysaccharide preparations may have been selected based on a low anti-IIa activity. The compositions of the invention include polysaccharide compositions designed to have either a high or low anti-IIa activity without regards to the sequence. The compositions of the invention include polysaccharide compositions designed to have a high anti-IIa activity and sequence specific low anti-IIa activity and methods of using these compositions. For instance, compositions having higher anti-IIa activity (e.g., M118 and M312) are more potent for indications such as arterial thrombosis (including ST elevation, MI and acute coronary syndrome (ACS)) than LMWHs which possess lower anti-IIa activity.

A "polysaccharide" is a polymer composed of monosaccharides linked to one another. In many polysaccharides the basic building block of the polysaccharide is actually a disaccharide unit, which can be repeating or non-repeating. Thus, a unit when used with respect to a polysaccharide refers to a basic building block of a polysaccharide and can include a monomeric building block (monosaccharide) or a dimeric building block (disaccharide).

It had been found that some polysaccharides have therapeutic activity. In particular, heparin is a widely used clinical anticoagulant. Heparin primarily elicits its effect through two mechanisms, both of which involve binding of antithrombin III (AT-III) to a specific pentasaccharide sequence, $H_{NAc/S,6S}GH_{NS,3S,6S}I_{2S}H_{NS,6S}$ contained within the polymer. First, AT-III binding to the pentasaccharide induces a conformational change in the protein that mediates its inhibition of factor Xa. Second, thrombin (factor Ia) also binds to heparin at a site proximate to the pentasaccharide AT-III binding site. Formation of a ternary complex between AT-III, thrombin and heparin results in inactivation of thrombin. Unlike its anti-Xa activity that requires only the AT-III pentasaccharide-binding site, heparin's anti-IIa activity is size-dependant, requiring at least 18 saccharide units for the efficient formation of an AT-III, thrombin, and heparin ternary complex. Additionally, heparin also controls the release of TFPI through binding of heparin to the endothelium lining the circulation system. Favourable release of TFPI, a modulator of the extrinsic pathway of the coagulation cascade, also results in further anticoagulation. In addition to heparin's anticoagulant properties, its complexity and wide distribution in mammals have lead to the suggestion that it may also be involved in a wide range of additional biological activities.

Although heparin is highly efficacious in a variety of clinical situations and has the potential to be used in many others, the side effects associated with heparin therapy are many and varied. Side effects such as heparin-induced thrombocytopenia (HIT) are primarily associated with the long chain of unfractionated heparin (UFH), which provides binding domains for various proteins. This has led to the generation and utilisation of low molecular weight heparin (LMWH) as an efficacious alternative to UFH. As a result, numerous strategies have been designed to create novel LMWHs with reduced chain lengths and fewer side effects. Of particular interest is the design of LMWHs that constitute the most active biological fragments of heparin. Examples of biologically active portions of a polysaccharide include but are not limited to a tetrasaccharide of the AT-III biding domain of heparin, a tetrasaccharide of the FGF biding domain of heparin, $I/GH_{NAC,6S}GH_{NS,3S,6S}$) $I/GUH_{NS,6S}GH_{NS,3S,6S}$, $I/GUH_{NAC,6S}GH_{NS,3S}$, or $I/GUH_{NS,6S}GH_{NS,3S}$. In other aspects, it is of interest to design LMWHs that have reduced portions that have or are associated with an unwanted biological activity, e.g., PF4 binding, e.g., $\Delta U_{2S}H_{NS,6S}$ (peak 1); $\Delta U_{2S}H_{NS}$(peak 2); $\Delta U_{2S}H_{NAC,6S}$ (peak 4); and/or $\Delta U_{2S}H_{NAC}$ (peak 6).

Sulfated polysaccharide preparations having structural and functional properties similar to LMWHs have been constructed and have been found to possess anti-Xa and anti-IIa activity as well as to promote the release of TFPI. Because of these attributes, the structure of these novel sulfated polysaccharide preparations could be assessed in conjunction with the beneficial activity. As shown below, the novel sulfated polysaccharide preparations of the invention demonstrate increased anti-Xa and anti-IIa activity or reduced Ia activity as well as TFPI release relative to UFH and other LMWHs. These novel LMWHs, likewise, contain a higher mole % of peak 8. It has also been found that the mole % of peak 8 is linearly correlated with anti-Xa and anti-IIa activity. It has also been shown that the novel polysaccharides have reduced PF4 binding activity. These novel LMWH have a lower mole % of $\Delta U_{2S}H_{NS,6S}$ (peak 1); $\Delta U_{2S}H_{NS}$(peak 2); $\Delta U_{2S}H_{NAC,6S}$ (peak 4); and/or $\Delta U_{2S}H_{NAC}$ (peak 6).

Mole % of a polysaccharide (e.g., a tetrasaccharide, a trisaccharide, a disaccharide, etc.) in this invention refers to the percentage of the number of moles of the polysaccharide in the sample, where one mole is 6.02×10^{23} molecules. In other words, mole % is also simply the number of molecules of the polysaccharide divided by the number of molecules present in the sample multiplied by 100.

It has also been discovered that the presence of the tetrasaccharide in the non-reducing end of the heparin sequence results in high anti-IIa activity. In the past, it was believed that this positioning of the tetrasaccharide would result in a composition having low anti-IIa activity. Compositions have been developed herein that have a predominant amount of the tetrasaccharide in the non-reducing end of the heparin sequence and have high anti-IIa activity.

Therefore, the invention relates to compositions of sulfated polysaccharides containing a useful amount of a beneficial feature such as a tetrasaccharide fragment represented by peak 8 and methods of treatment using compositions comprising peak 8.

Polysaccharide mixtures containing heterogeneous populations of heparin sequences can be fractionated into heparin of a specific size by varying the conditions described herein for temperature, solvent, and enzyme. The LMWH obtained by this procedure has high activity for anticoagulation, and low amount of the highly sulfated disaccharide represented by peak 1 (<70 mole %). In general, the higher molecular weight and/or higher charge fraction will precipitate at higher temperature, with a lower amount of polar solvent such as ethanol or acetone. Decreasing the temperature, and/or increasing the amount of polar solvent may result in the precipitation of the fraction with lower molecular weight, lower charge, and higher anticoagulation activity. Using the methods disclosed herein, the precipitation parameters may be altered without undue experimentation by one of ordinary skill in the art to obtain a preparation that conforms with the desired product.

Following the selective precipitation, the second fraction, the LMWH fraction, is processed to produce a sulfated polysaccharide preparation containing a specific amount of the tetrasaccharide represented by peak 8 as defined earlier. The processing step may involve an enzymatic or chemical digestion to yield the concentrated tetrasaccharides useful in the sulfated polysaccharide preparation. In one embodiment, the fraction is digested and the enzyme used in the digestion is Heparinase I or a functionally active variant or fragment thereof. In another embodiment the fraction is digested and

the enzyme used in the digestion is Heparinase II or a functionally active variant or fragment thereof. In another embodiment, the fraction is digested and the enzyme used in the digestion is Heparinase III or a functionally active variant or fragment thereof. In another embodiment, the fraction is digested and the enzyme used in the digestion is Heparinase IV or a functionally active variant or fragment thereof. In another embodiment, the fraction is digested and the enzyme used in the digestion is mammalian Heparanase or a functionally active variant or fragment thereof. In yet another embodiment, the fraction is digested and the enzyme used in the digestion is a mixture of one or more of Heparinase I, II, III, IV and Heparanase or a functionally active variant or fragment thereof. The term "heparinase" is used generically to encompass functionally active variants and fragments thereof in addition to the native heparinases, and includes bacterial and recombinant heparinases I, II, III, IV and heparanase, among others. Several patents and patent applications describe useful modifications and variants and fragments of heparinase, including U.S. Pat. No. 6,217,863 B1 and pending applications Ser. Nos. 09/384,959 and 09/802,285. Heparinase (as defined above) causes depolymerization of heparin. Depending upon the concentration of heparinase used, and the period for which it is used (partial vs exhaustive digestion), heparin of specific molecular weight, and/or charge is obtained. As an example, which is not intended to be limiting, a partial digestion of heparin with 1 molar equivalent of heparinase would result in a fraction of higher molecular weight, and/or higher charge than would a reaction with a longer digestion time. Also, increasing the molar equivalence of heparinase will result in a fraction with lower molecular weight and/or lower charge than if a lower molar equivalence of heparinase is used. In some embodiments, heparinase concentrations and length of digestions can be used in combination with salt, temperature, and solvent composition, as described herein, to obtain heparin of specific molecular weight, charge and/or biological activity.

Alternatively, following the selective precipitation, the LMWH fraction may be chemically degraded to yield the concentrated sulfated polysaccharide preparation. The fraction can be chemically degraded using a method selected from the group including but not limited to: oxidative depolymerization with H_2O_2 or Cu^+ and H_2O_2, deaminative cleavage with isoamyl nitrite, or nitrous acid, β-eliminative cleavage with benzyl ester of heparin by alkaline treatment or by heparinase.

Alternatively, the tetrasaccharide/peak 8 containing sequences may be produced synthetically. Examples of methods for synthesizing polysaccharides synthetically include U.S. patent application Ser. No. 60/263,621, filed Jan. 23, 2001, entitled: "Solid- and Solution-phase Synthesis of Heparin and Other Glycosaminoglycans" and U.S. patent application Ser. No. 09/413,381, filed on Oct. 6, 1999, entitled: "Synthesis Of Oligosaccharides In Solution And On The Solid Support" by Obadiah J. Plante and Peter H. Seeberger, the entire contents of which are incorporated by reference.

The sulfated polysaccharides may in some embodiments be substantially pure. As used herein, the term "substantially pure" means that the polysaccharides are essentially free of other substances to an extent practical and appropriate for their intended use. In particular, the polysaccharides are sufficiently pure and are sufficiently free from other biological constituents of their hosts environments, e.g., having less than 20%, 15%, 10%, 5%, 2%, or 1% of other biological constituents from the host environment, so as to be useful in, for example, producing pharmaceutical preparations.

In some cases the composition, whether substantially pure or not, may also include other compounds such as one or more heparin molecules. As used herein the term "heparin" refers to polysaccharides having heparin-like structural and functional properties. Heparin includes, but is not limited to, native heparin, low molecular weight heparin (LMWH), heparin, biotechnologically prepared heparin, chemically modified heparin, synthetic heparin, and heparan sulfate. The term "biotechnological heparin" or "biotechnologically prepared heparin" encompasses heparin that is prepared from natural sources of polysaccharides which have been chemically modified and is described in Razi et al., Bioche. J. 1995 Jul. 15; 309 (Pt 2): 465-72. Chemically modified heparin is described in Yates et al., Carbohydrate Res 1996 Nov. 20; 294:15-27, and is known to those of skill in the art. Synthetic heparin is well known to those of skill in the art and is described in Petitou, M. et al., Bioorg Med Chem Lett. 1999 Apr. 19; 9(8):1161-6. Native heparin is heparin derived from a natural source (such as porcine intestinal mucosa).

The compositions of this invention may also be formulated with additives. An "additive" as used herein may be a carrier molecule. These additives may or may not have biological activity. In the instance where the additives elicit biological activity, the activity may be complementary. That is, it may be useful for the same therapeutic purpose as the sulfated polysaccharide preparation. Additives may also have some specific function, such as tumor cell growth inhibition, but in general it is preferable that the additive not have a conflicting effect on the coagulation cascade. These additives may be polysaccharides such as dermatan sulfate, heparan sulfate and chondroitin sulfate and/or proteins, such as albumin. Other additives are known to those of skill in the art.

As shown below, the sulfated polysaccharides produced according to the invention have improved functional properties over prior art heparin and LMWH preparations. The ability to prepare a composition having a specific minimum amount of a structural signature output, e.g., the tetrasaccharides $I/GH_{NAC,6S}GH_{NS,3S,6S}$, (represented by $\Delta UH_{NAC,6S}GH_{NS,3S,6S}$) $I/GH_{NS,6S}GH_{NS,3S,6S}$ (represented by $\Delta UH_{NS,6S}GH_{NS,3S,6S}$); $I/GH_{NAC,6S}GH_{NS,3S}$ (represented by $\Delta UH_{NAC,6S}GH_{NS,3S}$); or $I/GH_{NS,6S}GH_{NS,3S}$ (represented by $\Delta UH_{NS,6S}GH_{NS,3S}$)(or related compounds), is advantageous because these compositions have dramatically improved therapeutic properties. The ability to prepare a composition having a specific maximum amount of a structural signature output, e.g., $\Delta U_{2S}H_{NS,6S}$ (peak 1), $\Delta U_{2S}H_{NS}$(peak 2), $\Delta U_{2S}H_{NAC,6S}$ (peak 4), and/or $\Delta U_{2S}H_{NAC}$ (peak 6), is advantageous because these compositions have reduced PF4 binding and this reduced likelihood of causing HIT. Thus, the compositions of the invention may include a preparation that has a structural signature very similar to that of a commercially available LMWH preparation such as enoxaparin, with an improvement, e.g., the addition of a desirable element, an increase in a desirable element, a decrease in an undesirable element, the elimination of an undesirable element and/or a reduction in batch to batch variability.

The structure of polysaccharides which are useful in the methods of the invention can be identified using techniques known in the art. The sequence of several polysaccharides has been identified using a property-encoded nomenclature/mass spectrometry scheme (PEN-MALDI), a sequencing methodology described in U.S. patent application Ser. Nos. 09/557,997 and 09/558,137 filed on Apr. 24, 2000, which are incorporated herein by reference in their entirety, and Venkataraman, G., Shriver, Z., Raman, R. & Sasisekharan, R. (1999) *Science* 286, 537-42. Using these techniques, the characteristics of a polysaccharide can be identified by any means

which is consistent with the experimental constraint used. Molecular weight may be determined by several methods including mass spectrometry. The use of mass spectrometry for determining the molecular weight of polysaccharides is well known in the art. Mass spectrometry has been used as a powerful tool to characterize polysaccharides because of its accuracy (±1 Dalton) in reporting the masses of fragments generated (e.g., by enzymatic cleavage), and also because only pM sample concentrations are required. For example, matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) has been described for identifying the molecular weight of polysaccharide fragments in publications such as Rhomberg, A. J. et al, *PNAS, USA,* v. 95, p. 4176-4181 (1998); Rhomberg, A. J. et al, *PNAS, USA,* v. 95, p. 12232-12237 (1998); and Ernst, S. et. al., *PNAS, USA,* v. 95, p. 4182-4187 (1998), each of which is hereby incorporated by reference. Other types of mass spectrometry known in the art, such as, electron spray-MS, fast atom bombardment mass spectrometry (FAB-MS) and collision-activated dissociation mass spectrometry (CAD) can also be used to identify the molecular weight of the polysaccharide fragments.

The mass spectrometry data may be a valuable tool to ascertain information about the polysaccharide component isolated from natural sources or synthesized without further treatment or after the polysaccharide has undergone degradation with enzymes or chemicals. After a molecular weight of a polysaccharide is identified, it may be compared to molecular weights of other known polysaccharides (e.g., using the methods of U.S. patent application Ser. Nos. 09/557,997 and 09/558,137, which are incorporated herein by reference in their entirety). As shown in these patent applications, one technique for comparing molecular weights is to generate a mass line and compare the molecular weight of the unknown polysaccharide to the mass line to determine a subpopulation of polysaccharides which have the same molecular weight. A "mass line" is an information database, preferably in the form of a graph or chart which stores information for each possible type of polysaccharide having a unique sequence based on the molecular weight of the polysaccharide. Because mass spectrometry data indicates the mass of a fragment to 1 Da accuracy, a length may be assigned uniquely to a fragment by looking up a mass on the mass line. Further, it may be determined from the mass line that, within a fragment of a particular length higher than a disaccharide, there is a minimum of 4.02 Da different in masses indicating that two acetate groups (84.08 Da) replaced a sulfate group (80.06 Da). Therefore, a number of sulfates and acetates of a polysaccharide fragment may be determined from the mass from the mass spectrometry data and, such number may be assigned to the polysaccharide fragment. In addition to molecular weight, other properties of a polysaccharide may be determined to fully characterize the polymer.

In a preferred embodiment, capillary electrophoresis (CE) is used to identify the disaccharide/tetrasaccharides building blocks. CE is superior to SAX HPLC in oligosaccharide analysis for several reasons. CE is significantly more accurate and precise than traditional LC due to the fact that there is no peak broadening resulting from laminar flow (as is the case with LC). The use of CE allows for 100% mass balance of di- and oligosaccharides after heparinase digestion. As a result, it is possible to resolve all of the lower prevalence oligosaccharides that are responsible for many of the clinical characteristics of heparins. In addition, CE requires the injection of 20 to 100 fold smaller amounts of saccharides compared to LC (500 fmols or less vs. at least 10 pmoles for capillary LC). Also, due to a larger number of theoretical plates, the resolving power of CE is higher than LC enabling separation of unique products (isomers) that contain an identical number of sulfates over a short run time. Thus the use of CE makes it possible to resolve all 32 building blocks that make up heparins.

CE also affords an added degree of flexibility in terms of complementarity to other analytical methodologies, including MALDI MS. In a further embodiment, the method of the invention relates to the use of CE separation and analysis followed by off-line MALDI MS analysis to derive structural information in an iterative way using bioinformatics.

Finally, the methods of the invention include the use of several techniques, including MALDI-MS, ESI-MS, CE and NMR, in combination to corroborate findings with respect to the structural signature of oligosaccharides. The methods of the invention make it possible to isolate, identify, and assign all the saccharide products that arise in a CE electrophoretogram of both heparin and various low LMWHs, including various 3-O-sulfated saccharides which are crucial for certain therapeutic utilities.

A further advantage of the methods of the invention is sensitivity; the methods make it possible to detect and characterize heparin samples down to a concentration range of 0.2-1 mg/mL.

Once a polysaccharide sample is characterized, the activity may be assessed in vitro or in vivo. Methods of determining the activity of sulfated polysaccharide preparations was assessed and shown in the Examples below. It was found that these preparations possessed a higher mole % of peak 8. The mole % of peak 8 was shown to be a good predictor of anticoagulation activity as the mole % of the tetrasaccharides were linearly correlated to both anti-IIa and anti-Xa activity. Additionally in vivo experiments further described in the Examples demonstrated anti-Xa and anti-IIa activity as well as increased TFPI release. Therefore, the compositions of the invention may be constructed and assessed according to the content of peak 8 as well as other fragments which may prove to be biologically important, e.g., $\Delta U_{2S}H_{NS,6S}$ (peak 1); $\Delta U_{2S}H_{NS}$(peak 2); $\Delta U_{2S}H_{NAC,6S}$ (peak 4); and/or $\Delta U_{2S}H_{NAC}$ (peak 6) which are associated with PF4 binding. The molar amount of these fragments in a sample are indicative of desirable activity and can be used in compositions and methods of treatment for diseases as will be described below. Furthermore, the molar amounts of these fragments may be used to predict what biological activity and levels of activity a given heparin compound will have, without the need for performing direct biological assays; thus, the method provides a way to both streamline manufacturing and reduce costs while ensuring a more consistent, higher quality product.

This information may be used to determine bioequivalence as well; by the following method, which is intended solely as an example and is not meant to be limiting. First, a reference standard is selected, and information about the composition and biological activity of a drug, e.g., how it is used and cleared by the body, is either provided or determined. They can be determined by any method, including the methods of the invention. The reference standard may be a previously characterized composition, or a new reference standard may be determined. Taking a LMWH preparation as an example, the reference standard would include information regarding the absorption of the preparation into the body; the clearance rates of the preparation out of the body; and the structural signature of the preparation. The same information is either provided or determined for one or more target compositions, and the two (or more) are compared; bioequivalence is determined by the variance between the two. Thus, the invention also relates to a method for determining bioequivalence.

The compositions may be administered therapeutically to a subject. As used herein, a "subject" is a human or non-human vertebrate such as a non-human primate, cow, horse, pig, sheep, goat, dog, cat, or rodent.

The compositions of the invention have many therapeutic utilities, and generally may be used for the treatment of any type of condition in which heparin, LMWH, or synthetic heparin therapy has been identified as a useful therapy. For instance, the invention includes methods for treating or preventing wherein the subject has or is at risk of a disorder selected from the group consisting of disease associated with coagulation, such as thrombosis, cardiovascular disease, vascular conditions or atrial fibrillation; migraine, atherosclerosis; an inflammatory disorder, such as autoimmune disease or atopic disorders; an allergy; a respiratory disorder, such as asthma, emphysema, adult respiratory distress syndrome (ARDS), cystic fibrosis, or lung reperfusion injury; a cancer or metastatic disorder; an angiogenic disorder, such as neovascular disorders of the eye, osteoporosis, psoriasis, and arthritis, Alzheimer's; bone fractures such as hip fractures; or is undergoing or having undergone surgical procedure, organ transplant, orthopedic surgery, hip replacement, knee replacement, percutaneous coronary intervention (PCI), stent placement, angioplasty, coronary artery bypass graft surgery (CABG).

Thus, the invention is useful in a variety of in vitro, in vivo and ex vivo methods in which LMWH therapies are useful. For instance, it is known that LMWH compositions are useful for preventing coagulation, inhibiting cancer cell growth and metastasis, preventing angiogenesis, preventing neovascularization, and preventing psoriasis. Each of these disorders is well-known in the art and is described, for instance, in *Harrison 's Principles of Internal Medicine* (McGraw Hill, Inc., New York), which is incorporated herein by reference.

When an imbalance in the coagulation pathway shifts towards excessive coagulation, the result is the development of thrombotic tendencies, which are often manifested as heart attacks, strokes, deep venous thrombosis, acute coronary syndrome, unstable angina and myocardial infarcts. A "disease associated with coagulation" as used herein refers to a condition characterized by local inflammation which may result in an interruption or reduction in the blood supply to a tissue which may occur, for instance, as a result of blockage of a blood vessel responsible for supplying blood to the tissue such as is seen for myocardial or cerebral infarction or peripheral vascular disease, or as a result of emboli formation associated with conditions such as atrial fibrillation or deep venous thrombosis. Coagulation disorders include, but are not limited to, cardiovascular disease and vascular conditions such as cerebral ischemia.

The methods are useful for treating cardiovascular disease. Cardiovascular diseases include, but are not limited to, acute myocardial infarction, unstable angina, acute coronary syndrome and atrial fibrillation. Myocardial infarction is a disease state which sometimes occurs with an abrupt decrease in coronary blood flow that follows a thrombotic occlusion of a coronary artery previously narrowed by atherosclerosis. Such injury may be produced or facilitated by factors such as cigarette smoking, hypertension, and lipid accumulation. Acute angina is due to transient myocardial ischemia. This disorder is usually associated with a heaviness, pressure, squeezing, smothering, or choking feeling below the sternum. Episodes are usually caused by exertion or emotion, but can occur at rest.

Atrial fibrillation is a common form of arrhythmia generally arising as a result of emotional stress or following surgery, exercise, or acute alcoholic intoxication. Persistent forms of atrial fibrillation generally occur in patients with cardiovascular disease. Atrial fibrillation is characterized by disorganized atrial activity without discrete P waves on the surface ECG.

Persons undergoing surgery, anesthesia and extended periods of bed rest or other inactivity are often susceptible to a condition known as deep venous thrombosis, or DVT, which is a clotting of venous blood in the lower extremities and/or pelvis. This clotting occurs due to the absence of muscular activity in the lower extremities required to pump the venous blood (stasis), local vascular injury or a hypercoagulable state. The condition can be life-threatening if a blood clot migrates to the lung, resulting in a "pulmonary embolus" or otherwise interferes with cardiovascular circulation. One method of treatment involves administration of an anti-coagulant.

The rapid absorption of biological agents, such as UFH or LMWH, after inhalation as dry particles can be very valuable in the treatment of myocardial infarction, acute coronary syndrome, and/or venous thromboembolism. Intravenous administration of UFH has been used widely for treatment of venous thromboembolism in combination with oral warfarin. Due to the improved efficacy and reduced risks, however, LMWHs have been increasingly used as an alternative to intravenous UFH in treatment of venous thromboembolism. The efficacy of heparin therapy may depend on achieving critical therapeutic levels (e.g., such as those that may be measured by anti-Xa activity and/or anti-IIa activity) within the first 24 hours of treatment. Intrapulmonary delivery of heparin particles to achieve rapid therapeutic levels of heparin in the early stage of thromboembolism, could also be combined with either s.c. administration of LMWHs or formulated heparin particles for prolonged antithrombotic/anticoagulant effect.

The methods of the invention are useful also for treating cerebral ischemia. A cerebral ischemic attack or cerebral ischemia is a form of ischemic condition in which the blood supply to the brain is blocked. This interruption in the blood supply to the brain may result from a variety of causes, including an intrinsic blockage or occlusion of the blood vessel itself, a remotely originated source of occlusion, decreased perfusion pressure or increased blood viscosity resulting in inadequate cerebral blood flow, or a ruptured blood vessel in the subarachnoid space or intracerebral tissue. Cerebral ischemia may result in either transient or permanent deficits and the seriousness of the neurological damage in a patient who has experienced cerebral ischemia depends on the intensity and duration of the ischemic event. A transient ischemic attack (TIA) is one in which the blood flow to the brain is interrupted only briefly and causes temporary neurological deficits, which often are clear in less than 24 hours. Symptoms of TIA include numbness or weakness of face or limbs, loss of the ability to speak clearly and/or to understand the speech of others, a loss of vision or dimness of vision, and a feeling of dizziness. Permanent cerebral ischemic attacks, also called stroke, are caused by a longer interruption in blood flow to the brain resulting from either a thrombus or thromboembolism. A stroke causes a loss of neurons typically resulting in a neurologic deficit that may improve but that does not entirely resolve. Thromboembolic stroke is due to the occlusion of an extracranial or intracranial blood vessel by a thrombus or embolus. Because it is often difficult to discern whether a stroke is caused by a thrombosis or an embolism, the term "thromboembolism" is used to cover strokes caused by either of these mechanisms. The methods of this invention also encompass treatment and prevention of thromboembolic complications that may develop post prosthesis surgery.

The compositions of the invention are also useful for treating or preventing wherein the subject has or is at risk of a disorder selected from the group consisting of disease associated with coagulation, such as thrombosis, cardiovascular disease, vascular conditions or atrial fibrillation; migraine, atherosclerosis; an inflammatory disorder, such as autoimmune disease or atopic disorders; an allergy; a respiratory disorder, such as asthma, emphysema, adult respiratory distress syndrome (ARDS), cystic fibrosis, or lung reperfusion injury; a cancer or metastatic disorder; an angiogenic disorder, such as neovascular disorders of the eye, osteoporosis, psoriasis, and arthritis, Alzheimers'; bone fractures such as hip fractures; or is undergoing or having undergone surgical procedure, organ transplant, orthopedic surgery, hip replacement, knee replacement, percutaneous coronary intervention (PCI), stent placement, angioplasty, coronary artery bypass graft surgery (CABG).

The compositions of the invention are also useful in the treatment of inflammatory or allergic disorders, including respiratory diseases such as cystic fibrosis, asthma, allergy, emphysema, and adult respiratory distress syndrome (ARDS); lung reperfusion injury; ischemia-reperfusion injury of the lung, kidney, heart, and gut; and lung tumor growth and metastasis.

Cystic fibrosis is a chronic progressive disease affecting the respiratory system. One serious consequence of cystic fibrosis is *Pseudomonas aeruginosa* lung infection, which by itself accounts for almost 90% of the morbidity and mortality in cystic fibrosis. Therapeutics for treating cystic fibrosis include antimicrobials for treating the pathogenic infection.

Asthma is a disorder of the respiratory system characterized by inflammation, narrowing of the airways and increased reactivity of the airways to inhaled agents. Asthma is frequently, although not exclusively, associated with atopic or allergic symptoms. Asthma may also include exercise induced asthma, bronchoconstrictive response to bronchostimulants, delayed-type hypersensitivity, auto immune encephalomyelitis and related disorders. Allergies are generally caused by IgE antibody generation against allergens. Emphysema is a distention of the air spaces distal to the terminal bronchiole with destruction of alveolar septa. Emphysema arises out of elastase induced lung injury. Bioactive agents such as heparin are capable of inhibiting this elastase induced injury. Adult respiratory distress syndrome is a term which encompasses many acute defuse infiltrative lung lesions of diverse ideologies which are accompanied by severe atrial hypoxemia. One of the most frequent causes of ARDS is sepsis. Other types of inflammatory diseases which are treatable are refractory ulcerative colitis, Crohn's disease, non-specific ulcerative colitis, multiple sclerosis, and interstitial cystitis.

The methods of the invention in some embodiments are directed to the treatment of acute thromboembolic stroke using sulfated polysaccharides. An acute stroke is a medical syndrome involving neurological injury resulting from an ischemic event, which is an interruption in the blood supply to the brain.

An effective amount of a sulfated polysaccharide preparation alone or in combination with another therapeutic for the treatment of stroke is that amount sufficient to reduce in vivo brain injury resulting from the stroke. A reduction of brain injury is any prevention of injury to the brain which otherwise would have occurred in a subject experiencing a thromboembolic stroke absent the treatment of the invention. Several physiological parameters may be used to assess reduction of brain injury, including smaller infarct size, improved regional cerebral blood flow, and decreased intracranial pressure, for example, as compared to pretreatment patient parameters, untreated stroke patients or stroke patients treated with thrombolytic agents alone.

The pharmaceutical sulfated polysaccharide preparation may be used alone or in combination with a therapeutic agent for treating a disease associated with coagulation. Examples of therapeutics useful in the treatment of diseases associated with coagulation include anticoagulation agents, antiplatelet agents, and thrombolytic agents.

Anticoagulation agents prevent the coagulation of blood components and thus prevent clot formation. Anticoagulants include, but are not limited to, heparin, warfarin, coumadin, dicumarol, phenprocoumon, acenocoumarol, ethyl biscoumacetate, hirudin, bivalarutin, and other direct thrombin inhibitors, and indandione derivatives.

Antiplatelet agents inhibit platelet aggregation and are often used to prevent thromboembolic stroke in patients who have experienced a transient ischemic attack or stroke. Antiplatelet agents include, but are not limited to, aspirin, thienopyridine derivatives such as ticlopodine and clopidogrel, dipyridamole and sulfinpyrazone, as well as RGD mimetics.

Thrombolytic agents lyse clots which cause the thromboembolic stroke. Thrombolytic agents have been used in the treatment of acute venous thromboembolism and pulmonary emboli and are well known in the art (e.g. see Hennekens et al, *J Am Coll Cardiol*; v. 25 (7 supp), p. 18S-22S (1995); Holmes, et al, *J Am Coll Cardiol*; v. 25 (7 suppl), p. 10S-17S (1995)). Thrombolytic agents include, but are not limited to, plasminogen, a_2-antiplasmin, streptokinase, antistreplase, TNK, tissue plasminogen activator (tPA), and urokinase. "tPA" as used herein includes native tPA and recombinant tPA, as well as modified forms of tPA that retain the enzymatic or fibrinolytic activities of native tPA. The enzymatic activity of tPA can be measured by assessing the ability of the molecule to convert plasminogen to plasmin. The fibrinolytic activity of tPA may be determined by any in vitro clot lysis activity known in the art, such as the purified clot lysis assay described by Carlson, et al., *Anal. Biochem.* 168, 428-435 (1988) and its modified form described by Bennett, W. F. et al, 1991, supra, the entire contents of which are hereby incorporated by reference.

In one embodiment, the sulfated polysaccharide preparations are used for inhibiting angiogenesis. An effective amount for inhibiting angiogenesis of the sulfated polysaccharide preparation is administered to a subject in need of treatment thereof. Angiogenesis as used herein is the inappropriate formation of new blood vessels. "Angiogenesis" often occurs in tumors when endothelial cells secrete a group of growth factors that are mitogenic for endothelium causing the elongation and proliferation of endothelial cells which results in a generation of new blood vessels. Several of the angiogenic mitogens are heparin binding peptides which are related to endothelial cell growth factors. The inhibition of angiogenesis can cause tumor regression in animal models, suggesting a use as a therapeutic anticancer agent. An effective amount for inhibiting angiogenesis is an amount of sulfated polysaccharide preparation which is sufficient to diminish the number of blood vessels growing into a tumor. This amount can be assessed in an animal model of tumors and angiogenesis, many of which are known in the art.

The sulfated polysaccharide preparations are also useful for inhibiting neovascularization associated with eye disease. In another embodiment, the sulfated polysaccharide preparation is administered to treat psoriasis. Psoriasis is a common dermatologic disease causes by chronic inflammation.

Sulfated polysaccharide containing compositions, may also inhibit cancer cell growth and metastasis. Thus the methods of the invention are useful for treating and/or preventing tumor cell proliferation, angiogenesis or metastasis in a subject. The terms "prevent" and "preventing" as used herein refer to inhibiting completely or partially the biological effect, e.g., angiogenesis or proliferation or metastasis of a cancer or tumor cell, as well as inhibiting any increase in the biological effect, e.g., angiogenesis or proliferation or metastasis of a cancer or tumor cell.

Cancers or tumors include but are not limited to biliary tract cancer; brain cancer; breast cancer; cervical cancer; choriocarcinoma; colon cancer; endometrial cancer; esophageal cancer; gastric cancer; intraepithelial neoplasms; leukemias, lymphomas; liver cancer; lung cancer (e.g. small cell and non-small cell); melanoma; neuroblastomas; oral cancer; ovarian cancer; pancreatic cancer; prostate cancer; rectal cancer; sarcomas; skin cancer; testicular cancer; thyroid cancer; and renal cancer, as well as other carcinomas and sarcomas.

A subject in need of cancer treatment may be a subject who has a high probability of developing cancer. These subjects include, for instance, subjects having a genetic abnormality, the presence of which has been demonstrated to have a correlative relation to a higher likelihood of developing a cancer and subjects exposed to cancer-causing agents such as tobacco, asbestos, or other chemical toxins, or a subject who has previously been treated for cancer and is in apparent remission.

When administered to a patient undergoing cancer treatment, the polysaccharide particles may be administered in cocktails containing other anti-cancer agents. The polysaccharide compositions may also be administered in cocktails containing agents that treat the side-effects of radiation therapy, such as anti-emetics, radiation protectants, etc.

Subjects in need of treatment may also be subjects with abnormal renal function, including renal failure, as measured by RFI, urea, creatinine, phosphorus, glomerular filtration rate (GFR), or blood urea nitrogen (BUN) levels in blood and/or urine. The specific measures are as follows:

Renal Failure Index (RFI)—in mEq/L is calculated as follows:

(urine sodium in mEq/L)/((urine creatinine in mg/dL)/(plasma creatinine in mg/dL)).

An RFI of ≦1 indicates prerenal azotemia; an RFI=1-3 is less definitive but usually indicates tubular necrosis; and an RFI≧3 indicates acute tubular necrosis

Urine Specific Gravity—This is a measure of how concentrated a urine sample is. Water has a specific gravity of 1.000. A dilute urine sample has a specific gravity less that 1.020 (often less than 1.010). A concentrated urine sample would have a specific gravity over 1.030 or 1.040.

Blood Urea Nitrogen (BUN)—This is a protein metabolite excreted by the kidney (it is one of the toxins we are concerned about). In a normal patient the BUN is 25 or so. A good goal for BUN in kidney failure is 60-80. Often at the time of diagnosis, BUN is well over 150, 200, or even 300.

Creatinine—This is another protein metabolite (though this one is less dependent on dietary protein intake than is BUN). A normal creatinine is less than 2.0. A good goal in kidney failure is a creatinine of 4.5 or less. BUN and creatinine may be tracked (together with several other parameters) over time and in response to different treatments.

Phosphorus—The calcium/phosphorus balance becomes deranged in kidney failure due to hormone changes that ensue as well as the inability of the failing kidney to excrete phosphorus. If calcium and phosphorus levels become too high, the soft tissues of the body will develop mineralized deposits which are inflammatory and uncomfortable. The bones will weaken as well.

Potassium—The failing kidney is unable to conserve potassium efficiently and supplementation may be needed.

Packed Cell Volume/Hematocrit—This is a measure of red blood cell amount. More literally it represents the percentage of the blood made up by red blood cells. The hormone that stimulates the production of red blood cells is made by the kidney. The failing kidney does not make this hormone in normal amounts and anemia can result. Anemia is often worsened by the extra fluid administrations needed to manage the kidney toxins.

Glomerular Filtration Rate (GFR)—This test is a measure of how well the kidneys are removing wastes and excess fluid from the blood. It may be calculated from the serum creatinine level using age, weight, gender and body size. Normal GFR can vary according to age, decreasing in aging subjects. The normal value for GFR is 90 or above. A GFR below 60 is a sign that the kidneys are not working properly. A GFR below 15 indicates probable kidney failure.

Disorders associated with abnormal renal function/failure include, but are not limited to, end stage nephritises, renal calculus, ischemia renal disease, hypertension nephropathy, diabetes nephropathy, glomerulonephritises, tubulointerstial nephritises, and renal hypertension.

Effective amounts of the composition containing sulfated polysaccharides of the invention are administered to subjects in need of such treatment. Effective amounts are those amounts which will result in a desired reduction in cellular proliferation or metastasis or prevent coagulation or other therapeutic benefit without causing other medically unacceptable side effects. Such amounts can be determined with no more than routine experimentation. It is believed that doses ranging from 1 nanogram/kilogram to 100 milligrams/kilogram, depending upon the mode of administration, will be effective. The effective percentage of intact sulfated polysaccharide may be determined with no more than routine experimentation. The absolute amount will depend upon a variety of factors (including whether the administration is in conjunction with other methods of treatment, the number of doses and individual patient parameters including age, physical condition, size and weight) and can be determined with routine experimentation. It is preferred generally that a maximum dose be used, that is, the highest safe dose according to sound medical judgment. The mode of administration may be any medically acceptable mode including inhalation, oral, subcutaneous, intravenous, intraperitoneal, transdermal, buccal, sublingual, parenteral, intramuscular, intranasal, intratracheal, ocular, vaginal, rectal, transdermal, and/or sublingual.

In some aspects of the invention, the effective amount of a composition containing sulfated polysaccharide is that amount effective to prevent invasion of a tumor cell across a barrier. The invasion and metastasis of cancer is a complex process which involves changes in cell adhesion properties which allow a transformed cell to invade and migrate through the extracellular matrix (ECM) and acquire anchorage-independent growth properties. Liotta, L. A., et al., Cell 64:327-336 (1991). Some of these changes occur at focal adhesions, which are cell/ECM contact points containing membrane-associated, cytoskeletal, and intracellular signaling molecules. Metastatic disease occurs when the disseminated foci of tumor cells seed a tissue which supports their growth and propagation, and this secondary spread of tumor cells is responsible for the morbidity and mortality associated with the majority of cancers. Thus the term "metastasis" as used

herein refers to the invasion and migration of tumor cells away from the primary tumor site.

The barrier for the tumor cells may be an artificial barrier in vitro or a natural barrier in vivo. In vitro barriers include but are not limited to extracellular matrix coated membranes, such as Matrigel. Thus, the LMWH compositions can be tested for their ability to inhibit tumor cell invasion in a Matrigel invasion assay system as described in detail by Parish, C. R., et al., "A Basement-Membrane Permeability Assay which Correlates with the Metastatic Potential of Tumour Cells," Int. J. Cancer (1992) 52:378-383. Matrigel is a reconstituted basement membrane containing type IV collagen, laminin, heparan sulfate proteoglycans such as perlecan, which bind to and localize bFGF, vitronectin as well as transforming growth factor (TGF), urokinase-type plasminogen activator (uPA), tissue plasminogen activator (tPA), and the serpin known as plasminogen activator inhibitor type 1 (PAI-1). Other in vitro and in vivo assays for metastasis have been described in the prior art, see, e.g., U.S. Pat. No. 5,935,850, issued on Aug. 10, 1999, which is incorporated herein by reference. An in vivo barrier refers to a cellular barrier present in the body of a subject.

The sulfated polysaccharides of the invention may optionally be formulated in a pharmaceutically acceptable carrier. The compositions may further be formulated into specific delivery devices. Thus, in some embodiments of the invention the compositions are specifically formulated for intravenous, subcutaneous, oral, aerosol, or other mucosal form of delivery. In some embodiments the compositions are formulated in sustained release devices as described below.

In general, when administered for therapeutic purposes, the formulations of the invention are applied in pharmaceutically acceptable solutions. Such preparations may routinely contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, compatible carriers, adjuvants, and optionally other therapeutic ingredients.

The compositions of the invention may be administered per se (neat) or in the form of a pharmaceutically acceptable salt. When used in medicine the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically acceptable salts thereof and are not excluded from the scope of the invention. Such pharmacologically and pharmaceutically acceptable salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulphuric, nitric, phosphoric, maleic, acetic, salicylic, p-toluene sulphonic, tartaric, citric, methane sulphonic, formic, malonic, succinic, naphthalene-2-sulphonic, and benzene sulphonic. Also, pharmaceutically acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts of the carboxylic acid group.

Suitable buffering agents include: acetic acid and a salt (1-2 mole % W/V); citric acid and a salt (1-3 mole % W/V); boric acid and a salt (0.5-2.5 mole % W/V); and phosphoric acid and a salt (0.8-2 mole % W/V). Suitable preservatives include benzalkonium chloride (0.003-0.03 mole % W/V); chlorobutanol (0.3-0.9 mole % W/V); parabens (0.01-0.25 mole % W/V) and thimerosal (0.004-0.02 mole % W/V).

The invention provides pharmaceutical compositions, for medical use, which comprise sulfated polysaccharide preparations together with one or more pharmaceutically acceptable carriers and optionally other therapeutic ingredients. The term "pharmaceutically-acceptable carrier" as used herein, and described more fully below, means one or more compatible solid or liquid filler, dilutants or encapsulating substances which are suitable for administration to a human or other animal. In the invention, the term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being commingled with the sulfated polysaccharide of the invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficiency.

Compositions suitable for parenteral administration conveniently comprise a sterile aqueous preparation of the polysaccharide, which can be isotonic with the blood of the recipient. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables. Carrier formulations suitable for subcutaneous, intramuscular, intraperitoneal, intravenous, etc. administrations may be found in Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa.

A variety of administration routes are available. The particular mode selected will depend, of course, upon the particular percentage of sulfated polysaccharide selected, the particular condition being treated, and the dosage required for therapeutic efficacy. The methods of this invention, generally speaking, may be practiced using any mode of administration that is medically acceptable, meaning any mode that produces effective levels of a biological effect without causing clinically unacceptable adverse effects.

For use in therapy, an effective amount of the sulfated polysaccharide preparation can be administered to a subject by any mode that delivers the sulfated polysaccharide to the desired surface, e.g., mucosal, systemic. "Administering" the pharmaceutical composition of the invention may be accomplished by any means known to the skilled artisan. Preferred routes of administration include, but are not limited to, inhalation, oral, subcutaneous, intravenous, intraperitoneal, transdermal, buccal, buccal, sublingual, parenteral, intramuscular, intranasal, intratracheal, ocular, vaginal, rectal, transderrnal, and/or sublingual.

For oral administration, the compounds (i.e., sulfated polysaccharide preparations) can be formulated readily by combining the active compound(s) with pharmaceutically acceptable carriers well-known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject to be treated. Pharmaceutical preparations for oral use can be obtained as solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Optionally, the oral formulations may also be formulated in saline or buffers for neutralizing internal acid conditions or may be administered without any carriers.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrroli-

done, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. Microspheres formulated for oral administration may also be used. Such microspheres have been well defined in the art. All formulations for oral administration should be in dosages suitable for such administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the invention may be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g. gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch. In addition, dry powder formations for inhalation therapy are within the scope of the invention. Such dry powder formulations may be prepared as disclosed in WO 02/32406, the entire teachings of which are incorporated herein by reference.

The compounds, when it is desirable to deliver them systemically, may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active compounds may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal or vaginal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long-acting formulations may be formulated with suitable polymeric or hydrophobic materials (for example, as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include, but are not limited to, calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

Suitable liquid or solid pharmaceutical preparation forms are, for example, aqueous or saline solutions for inhalation, microencapsulated, encochleated, coated onto microscopic gold particles, contained in liposomes, nebulized, aerosols, pellets for implantation into the skin, or dried onto a sharp object to be scratched into the skin. The pharmaceutical compositions also include granules, powders, tablets, coated tablets, (micro) capsules, suppositories, syrups, emulsions, suspensions, creams, drops or preparations with protracted release of active compounds, in whose preparation excipients and additives and/or auxiliaries such as disintegrants, binders, coating agents, swelling agents, lubricants, flavorings, sweeteners or solubilizers are customarily used as described above. The pharmaceutical compositions are suitable for use in a variety of drug delivery systems. For a brief review of methods for drug delivery, see Langer, *Science* 249:1527-1533, (1990), which is incorporated herein by reference.

The compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active sulfated polysaccharide into association with a carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the polysaccharide into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product. The polysaccharide may be stored lyophilized.

Other delivery systems can include time-release, delayed release or sustained release delivery systems. Such systems can avoid repeated administrations of the sulfated polysaccharide of the invention, increasing convenience to the subject and the physician. Many types of release delivery systems are available and known to those of ordinary skill in the art. They include polymer based systems such as polylactic and polyglycolic acid, polyanhydrides and polycaprolactone; nonpolymer systems that are lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono-, di and triglycerides; hydrogel release systems; silastic systems; peptide based systems; wax coatings, compressed tablets using conventional binders and excipients, partially fused implants and the like. Specific examples include, but are not limited to: (a) erosional systems in which the polysaccharide is contained in a form within a matrix, found in U.S. Pat. No. 4,452,775 (Kent); U.S. Pat. No. 4,667,014 (Nestor et al.); and U.S. Pat. Nos. 4,748,034 and 5,239,660 (Leonard) and (b) diffusional systems in which an active component permeates at a controlled rate through a polymer, found in U.S. Pat. No. 3,832,253 (Higuchi et al.) and U.S. Pat. No. 3,854,480 (Zaffaroni). In addition, a pump-based hardware delivery system can be used, some of which are adapted for implantation.

When administered to a patient undergoing cancer treatment, the sulfated polysaccharide compositions may be administered in cocktails containing other anti-cancer agents.

The compositions may also be administered in cocktails containing agents that treat the side-effects of therapy, such as anti-emetics, radiation protectants, etc.

Anti-cancer drugs that can be co-administered with the compounds of the invention include, but are not limited to Acivicin; Aclarubicin; Acodazole Hydrochloride; Acronine; Adriamycin; Adozelesin; Aldesleukin; Altretamine; Ambomycin; Ametantrone Acetate; Aminoglutethimide; Amsacrine; Anastrozole; Anthramycin; Asparaginase; Asperlin; Azacitidine; Azetepa; Azotomycin; Batimastat; Benzodepa; Bicalutamide; Bisantrene Hydrochloride; Bisnafide Dimesylate; Bizelesin; Bleomycin Sulfate; Brequinar Sodium; Bropirimine; Busulfan; Cactinomycin; Calusterone; Caracemide; Carbetimer; Carboplatin; Carmustine; Carubicin Hydrochloride; Carzelesin; Cedefingol; Chlorambucil; Cirolemycin; Cisplatin; Cladribine; Crisnatol Mesylate; Cyclophosphamide; Cytarabine; Dacarbazine; Dactinomycin; Daunorubicin Hydrochloride; Decitabine; Dexormaplatin; Dezaguanine; Dezaguanine Mesylate; Diaziquone; Docetaxel; Doxorubicin; Doxorubicin Hydrochloride; Droloxifene; Droloxifene Citrate; Dromostanolone Propionate; Duazomycin; Edatrexate; Eflornithine Hydrochloride; Elsamitrucin; Enloplatin; Enpromate; Epipropidine; Epirubicin Hydrochloride; Erbulozole; Esorubicin Hydrochloride; Estramustine; Estramustine Phosphate Sodium; Etanidazole; Etoposide; Etoposide Phosphate; Etoprine; Fadrozole Hydrochloride; Fazarabine; Fenretinide; Floxuridine; Fludarabine Phosphate; Fluorouracil; Flurocitabine; Fosquidone; Fostriecin Sodium; Gemcitabine; Gemcitabine Hydrochloride; Glevec; Herceptin; Hydroxyurea; Idarubicin Hydrochloride; Ifosfamide; Ilmofosine; Interferon Alfa-2a; Interferon Alfa-2b; Interferon Alfa-n1; Interferon Alfa-n3; Interferon Beta-I a; Interferon Gamma-I b; Iproplatin; Irinotecan Hydrochloride; Lanreotide Acetate; Letrozole; Leuprolide Acetate; Liarozole Hydrochloride; Lometrexol Sodium; Lomustine; Losoxantrone Hydrochloride; Masoprocol; Maytansine; Mechlorethamine Hydrochloride; Megestrol Acetate; Melengestrol Acetate; Melphalan; Menogaril; Mercaptopurine; Methotrexate; Methotrexate Sodium; Metoprine; Meturedepa; Mitindomide; Mitocarcin; Mitocromin; Mitogillin; Mitomalcin; Mitomycin; Mitosper; Mitotane; Mitoxantrone Hydrochloride; Mycophenolic Acid; Nocodazole; Nogalamycin; Ormaplatin; Oxisuran; Paclitaxel; Pegaspargase; Peliomycin; Pentamustine; Peplomycin Sulfate; Perfosfamide; Pipobroman; Piposulfan; Piroxantrone Hydrochloride; Plicamycin; Plomestane; Porfimer Sodium; Porfiromycin; Prednimustine; Procarbazine Hydrochloride; Puromycin; Puromycin Hydrochloride; Pyrazofurin; Riboprine; Rituxin; Rogletimide; Safingol; Safingol Hydrochloride; Semustine; Simtrazene; Sparfosate Sodium; Sparsomycin; Spirogermanium Hydrochloride; Spiromustine; Spiroplatin; Streptonigrin; Streptozocin; Sulofenur; Talisomycin; Tecogalan Sodium; Tegafur; Teloxantrone Hydrochloride; Temoporfin; Teniposide; Teroxirone; Testolactone; Thiamiprine; Thioguanine; Thiotepa; Tiazofurin; Tirapazamine; Topotecan Hydrochloride; Toremifene Citrate; Trestolone Acetate; Triciribine Phosphate; Trimetrexate; Trimetrexate Glucuronate; Triptorelin; Tubulozole Hydrochloride; Uracil Mustard; Uredepa; Vapreotide; Verteporfin; Vinblastine Sulfate; Vincristine Sulfate; Vindesine; Vindesine Sulfate; Vinepidine Sulfate; Vinglycinate Sulfate; Vinleurosine Sulfate; Vinorelbine Tartrate; Vinrosidine Sulfate; Vinzolidine Sulfate; Vorozole; Zeniplatin; Zinostatin; Zorubicin Hydrochloride.

The sulfated polysaccharide compositions may also be linked to a targeting molecule. A targeting molecule is any molecule or compound which is specific for a particular cell or tissue and which can be used to direct the sulfated polysaccharide to the cell or tissue. Preferably the targeting molecule is a molecule which specifically interacts with a cancer cell or a tumor. For instance, the targeting molecule may be a protein or other type of molecule that recognizes and specifically interacts with a tumor antigen.

Tumor antigens include but are not limited to Melan-A/MART-1, Dipeptidyl peptidase IV (DPPIV), adenosine deaminase-binding protein (ADAbp), cyclophilin b, Colorectal associated antigen (CRC)—C017-1A/GA733, Carcinoembryonic Antigen (CEA) and its immunogenic epitopes CAP-1 and CAP-2, etv6, aml1, Prostate Specific Antigen (PSA) and its immunogenic epitopes PSA-1, PSA-2, and PSA-3, prostate-specific membrane antigen (PSMA), T-cell receptor/CD3-zeta chain, MAGE-family of tumor antigens (e.g., MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A7, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, MAGE-Xp2 (MAGE-B2), MAGE-Xp3 (MAGE-B3), MAGE-Xp4 (MAGE-B4), MAGE-C1, MAGE-C2, MAGE-C3, MAGE-C4, MAGE-C5), GAGE-family of tumor antigens (e.g., GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7, GAGE-8, GAGE-9), BAGE, RAGE, LAGE-1, NAG, GnT-V, MUM-1, CDK4, tyrosinase, p53, MUC family, HER2/neu, p21ras, RCAS1, fetoprotein, E-cadherin, catenin, p120ctn, gp100^{Pmel117}, PRAME, NY-ESO-1, brain glycogen phosphorylase, SSX-1, SSX-2 (HOM-MEL-40), SSX-1, SSX-4, SSX-5, SCP-1, CT-7, cdc27, adenomatous polyposis coli protein (APC), fodrin, P1A, Connexin 37, Ig-idiotype, p15, gp75, GM2 and GD2 gangliosides, viral products such as human papilloma virus proteins, Smad family of tumor antigens, Imp-1, EBV-encoded nuclear antigen (EBNA)-1, and c-erbB-2.

Examples of tumor antigens which bind to either or both MHC class I and MHC class II molecules, see the following references: Coulie, *Stem Cells* 13:393-403, 1995; Traversari et al., J. Exp. Med. 176:1453-1457, 1992; Chaux et al., J. Immunol. 163:2928-2936, 1999; Fujie et al., Int. J. Cancer 80:169-172, 1999; Tanzarella et al., Cancer Res. 59:2668-2674, 1999; van der Bruggen et al., Eur. J. Immunol. 24:2134-2140, 1994; Chaux et al., J. Exp. Med. 189:767-778, 1999; Kawashima et al, Hum. Immunol. 59:1-14, 1998; Tahara et al., Clin. Cancer Res. 5:2236-2241, 1999; Gaugler et al., J. Exp. Med. 179:921-930, 1994; van der Bruggen et al., Eur. J. Immunol. 24:3038-3043, 1994; Tanaka et al., Cancer Res. 57:4465-4468, 1997; Oiso et al., Int. J. Cancer 81:387-394, 1999; Herman et al., Immunogenetics 43:377-383, 1996; Manici et al., J. Exp. Med. 189:871-876, 1999; Duffour et al., Eur. J. Immunol. 29:3329-3337, 1999; Zorn et al., Eur. J. Immunol. 29:602-607, 1999; Huang et al., J. Immunol.162: 6849-6854, 1999; Boël et al., Immunity 2:167-175, 1995; Van den Eynde et al., J. Exp. Med. 182:689-698, 1995; De Backer et al., Cancer Res. 59:3157-3165, 1999; Jäger et al., J. Exp. Med. 187:265-270, 1998; Wang et al., J. Immunol. 161:3596-3606, 1998; Aarnoudse et al., Int. J. Cancer 82:442-448, 1999; Guilloux et al., J. Exp. Med. 183:1173-1183, 1996; Lupetti et al., J. Exp. Med. 188:1005-1016, 1998; Wölfel et al., Eur. J. Immunol. 24:759-764, 1994; Skipper et al., J. Exp. Med. 183:527-534, 1996; Kang et al., J. Immunol. 155:1343-1348, 1995; Morel et al., Int. J. Cancer 83:755-759, 1999; Brichard et al., Eur. J. Immunol. 26:224-230, 1996; Kittlesen et al., J. Immunol. 160:2099-2106, 1998; Kawakami et al., J. Immunol. 161:6985-6992, 1998; Topalian et al., J. Exp. Med. 183:1965-1971, 1996; Kobayashi et al., Cancer Research 58:296-301, 1998; Kawakami et al., J. Immunol. 154:3961-3968, 1995; Tsai et al., J. Immunol. 158:1796-1802, 1997; Cox et al., Science 264:716-719, 1994; Kawakami et al.,

Proc. Natl. Acad. Sci. USA 91:6458-6462, 1994; Skipper et al., J. Immunol. 157:5027-5033, 1996; Robbins et al., J. Immunol. 159:303-308, 1997; Castelli et al, J. Immunol. 162:1739-1748, 1999; Kawakami et al., J. Exp. Med. 180: 347-352, 1994; Castelli et al., J. Exp. Med. 181:363-368, 1995; Schneider et al., Int. J. Cancer 75:451-458, 1998; Wang et al., J. Exp. Med. 183:1131-1140, 1996; Wang et al., J. Exp. Med. 184:2207-2216, 1996; Parkhurst et al., Cancer Research 58:4895-4901, 1998; Tsang et al., J. Natl Cancer Inst 87:982-990, 1995; Correale et al., J Natl Cancer Inst 89:293-300,1997; Coulie et al., Proc. Natl. Acad. Sci. USA 92:7976-7980, 1995; Wölfel et al., Science 269:1281-1284, 1995; Robbins et al., J. Exp. Med. 183:1185-1192, 1996; Brändle et al., J. Exp. Med. 183:2501-2508, 1996; ten Bosch et al., Blood 88:3522-3527, 1996; Mandruzzato et al., J. Exp. Med. 186:785-793, 1997; Guéguen et al., J. Immunol. 160: 6188-6194, 1998; Gjertsen et al., Int. J. Cancer 72:784-790, 1997; Gaudin et al., J. Immunol. 162:1730-1738, 1999; Chiari et al., Cancer Res. 59:5785-5792, 1999; Hogan et al., Cancer Res. 58:5144-5150, 1998; Pieper et al., J. Exp. Med. 189:757-765, 1999; Wang et al., Science 284:1351-1354, 1999; Fisk et al., J. Exp. Med. 181:2109-2117, 1995; Brossart et al., Cancer Res. 58:732-736, 1998; Röpke et al., Proc. Natl. Acad. Sci. USA 93:14704-14707, 1996; Ikeda et al., Immunity 6:199-208, 1997; Ronsin et al., J. Immunol. 163:483-490, 1999; Vonderheide et al., Immunity 10:673-679,1999. These antigens as well as others are disclosed in PCT Application PCT/US98/18601.

The following description of experiments performed is exemplary and non-limiting to the scope of the claimed invention.

EXAMPLES

Example 1

Development of a Compositional Analysis Method for the Structural Characterization of Heparins

Several techniques have been utilized for the structural analysis of heparin preparations. Gradient polyacrylamide gel electrophoresis (PAGE) and strong ion exchange HPLC (SAX) have previously been used for the qualitative and quantitative analysis of heparin preparations (Liu et al., *Glycobiology* 5:765-774, 1995; Turnbull et al., *Proc. Natl. Acad. Sci. USA* 96: 2698-2703, 1999; Merry et al., *J. Biol. Chem.* 274: 18455-18462, 1999). The gradient PAGE method, while useful in determining molecular weight, cannot offer information about the fine structure of heparin preparations. SAX-HPLC, relying on detection by ultraviolet absorbance or radioactivity, is often insufficiently sensitive for the detection of small amounts of structurally important heparin-derived oligosaccharides.

Capillary electrophoresis (CE) is a very sensitive methodology with high resolving power that has been used to characterize heparan sulfate-like glycosaminoglycan (HLGAG) oligosaccharides. In addition, CE has been used in conjunction with enzymatic digestion, to characterize the disaccharide composition of HLGAG complex oligosaccharides. See, e.g., U.S. patent application Ser. Nos. 09/557,997 and 09/558,137 filed on Apr. 24, 2000, which are incorporated herein by reference in their entirety. In combination with either off-line or on-line mass spectral analysis, CE is a powerful tool for the structural characterization and quantification of HLGAG di- and oligosaccharides.

The methods of the invention make it possible to complete the structural characterization of UFH and LMWHs using a similar approach. Determining the exact composition of UFHs from different sources as well as LMWHs is the first important step in correlating function with a structure. This is of special importance since current manufacturing practices for UFH and LMWHs use functional assays (e.g., anti-Xa assay) and gross physical characterization to provide quality control. Development of appropriate tools for the structural characterization of heparins would provide a more rigorous tool for the analysis of heparin.

This technique, viz., a Compositional Analysis Method (CAM), can be used to quantify the di- and tetrasaccharide and higher building blocks of heparins. Furthermore, this technique can be used to provide a key correlate between the structure and the function of various heparin and LMWH preparations, thus providing the guidance necessary to create compositions with a desired activity profile.

Materials and Methods

UFH was purchased from Celsus Laboratories (Cincinnati, Ohio) and molar concentrations of stocks were calculated based on an average molecular weight of 12,000 Da. Disaccharide standards were purchased from Sigma chemicals (St. Louis, Mo.). Heparinase I, II, and III were produced as described previously (Ernst, et al. *Biochem. J.* 315:589-597 (1996); Pojasek, et al. *Biochemistry* 39: 4012-4019 (2000)).

UFH was subjected to exhaustive depolymerization with an enzyme cocktail made up of heparinase I, II, and heparinase III. 9 μl of 10 μg/μl concentration of UFH in H_2O was digested with 1 μl of an enzyme cocktail consisting of 100 nM each of heparinase I, II, and III in 25 mM sodium acetate, 100 mM sodium chloride, 5 mM calcium acetate buffer, pH 7.0 for 12 hours at 37° C. The CE sample was prepared by diluting 1 μl of the digest with 9 μl of H_2O. Mass spectra were collected using parameters as outlined previously and calibrated externally by using signals for protonated $(RG)_{19}R$ and its complex with a nitrous acid-derived hexasaccharide of the sequence $I_{2S}H_{NS,6S}\,I_{2S}H_{NS,6S}\,I_{2S}Man_{6S}$.

Results

Development of CAM using UFH. As a first step towards the development of CAM, we analyzed UFH from a commercial source. UFH was subjected to exhaustive depolymerization with an enzyme cocktail made up of heparinases I, II, and III from *Flavobacterium heparinum*. Digestion of UFH with a heparinase cocktail results in cleavage of glycosidic linkages via the elimination of water resulting in the degradation of the polymer chain. The resulting products, with a $\Delta^{4,5}$ unsaturated uronic acid at the non-reducing end, readily absorb UV light and can be facially monitored at their λ_{max} of 232 nm.

To measure the accuracy and precision of the CAM technique, UFH was digested in duplicate and each sample was analyzed independently by CE. In addition, the experiment was repeated twice for each sample, resulting in four readings for UFH. Importantly, this entire analysis used only nanograms of starting material, ensuring the consumption of a minimum of material. Separation of the species resulting from enzymatic digestion of UFH by CE resulted in the appearance of eight distinct species that could be readily quantified (FIG. **1**). Capillary electrophoresis trace of UFH derived from porcine intestinal mucosa. 9 μl of 10 ng/nL concentration of UFH was digested with 1 μL of enzyme cocktail consisting of 100 nM each of Heparinase I, II, and III for 12 hours at 37° C. The CE sample was prepared by diluting the digest with H_2O to give a final heparin concentration of 1.0 ng/nL. 57 nL of this CE sample was injected into the CE. Each of the eight peaks p1-p8 was collected, the purity of the collected peaks was checked by re-injecting into

CE, and their mass was measured by offline MALDI Mass Spectrometry. The identity of p1-p7 was further confirmed by matching their migration time with that of standard, commercially available disaccharides. p1 was thus confirmed as the trisulfated disaccharide $\Delta U_{2S}H_{NS,6S}$. p2, p3, and p4 are disulfated disaccharides, and p5, p6, and 7 are monosulfated disaccharides. p8 was determined as a tetra or penta sulfated, non/mono acetylated tetrasaccharide comprising of one or more of the following: $\Delta UH_{NAc,6S}GH_{NS,3S,6S}$; $\Delta UH_{NS,6S}GH_{NS,3S,6S}$; $\Delta UH_{NAc,6S}GH_{NS,3S}$; or $\Delta UH_{NS,6S}GH_{NS,3S}$, referred to herein as "p8" or "peak 8." In a similar manner, peaks (in addition to the 8 peaks described here) present in other LMWH samples such as enoxaparin, and dalteparin have also been characterized. The species, labeled 1 through 8, were collected and characterized. First, the mass of each unknown was assessed by offline MALDI MS as described previously U.S. Pat. No. 5,607,859. Further, to confirm the identity of the unknown, the collected peak was desalted, rerun on CE and identified by comigration with known standards.

Consistent with previous structural studies on UFH and what is known of the substrate specificities of the heparinases, seven of the eight unknowns (p1-p7 of 8) were identified as disaccharides. Species p1 was identified as $\Delta U_{2S}H_{NS,6S}$, the primary disaccharide building block of heparin. Unknowns p2-p4 were identified as disulfated disaccharide isomers: $\Delta U_{2S}H_{NS}$, $\Delta UH_{NS,6S}$, $\Delta U_{2S}H_{NAc,6S}$, accordingly. Finally, p5-p7 were identified as the monosulfated disaccharides ΔUH_{NS}, $\Delta U_{2S}H_{NAc}$, $\Delta UH_{NAc,6S}$. Importantly, very little, if any, unsulfated disaccharide (ΔUH_{NAc}) could be detected in UFH.

In addition to the characterization of the seven disaccharides, we also completed structural characterization of unknown p8. Isolation and sequencing of this oligosaccharide using the PEN-MALDI sequencing approach (Venkataraman, et al., Science 286:537-42 (1999)) indicated that p8 is a tetra or penta sulfated, non/mono acetylated tetrasaccharide comprising of one or more of the following: $\Delta UH_{NAc,6S}GH_{NS,3S,6S}$; $\Delta UH_{NS,6S}GH_{NS,3S,6S}$; $\Delta UH_{NAc,6S}GH_{NS,3S}$; or $\Delta UH_{NS,6S}GH_{NS,3S}$. To quantify the mole % in heparin of p1-p8 requires the determination of the response factor (RF) for each species. To obtain the RF for each species, known concentrations of standards for p1-p8 were injected on the CE and used to determine a RF for each (Table 1). We then used these RFs to determine the mole % of each saccharide unit in heparin (Table 1). Analysis of the mole % composition of heparin indicates that most of the polymer chain (>50 mole %) consists of the trisulfated disaccharide: $\Delta U_{2S}H_{NS,6S}$. Another ≧20 mole % of the UFH chain consists of the different isomers of the disulfated disaccharides, with minor contributions from the monosulfated disaccharides and the tetrasaccharide of peak 8.

TABLE 1

Compositional Analysis Table for UFH

Compound	AUC	% Relative AUC	Response Factor (RF)	Corrected concentration	Mole %
p1	14639	62.1	1	62.1	66.1
p2	2050.9	8.7	0.893	7.8	8.3
p3	3088.1	13.1	0.829	10.9	11.6
p4	707.2	3	0.823	2.5	2.6
p5	1249.4	5.3	0.601	3.2	3.4
p6	895.8	3.8	0.405	1.5	1.6
p7	235.7	1.0	0.572	0.6	0.6
p8	707.2	3	1.768	5.3	5.6

The area under the curve (AUC) was measured for each peak from the CE spectrum of UFH digested with the enzyme cocktail as shown in FIG. **1**. The response factor calculated for each saccharide was used to calculate their corrected relative concentration in the enzyme digest. The last column gives the mole percentage of each of the building block of UFH. The unsulfated saccharides, which constitute <1 mole % of UFH, is not taken into consideration in constructing this compositional analysis table. As demonstrated here, construction of this compositional analysis table as shown by this method is independent of the concentration or the weight of the heparin digest analyzed by the CE.

Column 1 gives the AUC measured for p1-p8. Column 2 gives the % relative AUC. Multiplying the % relative AUC with the RF gives the corrected relative concentration or the % relative AUC of p1-p8 in terms of peak 1. These are then normalized to get the molar % of disaccharides p1-p7 and tetrasaccharide p8.

Experiments were completed to verify the instrumental reproducibility and to ascertain if the compositional analysis digest is indeed complete. There was little variability (less than 4%) in migration times and mole % determinations among samples, regardless of the sample amount injected into the capillary (varying over three orders of magnitude) or the amount of enzyme cocktail that was added to the sample (from 100 nM enzyme to 1 μM) (Table 2). Taken together, these results indicate that CAM is a rigorous, sensitive, and accurate methodology to determine the composition of UFH.

Sample	p1	p2	p3	p4	p5	p6	p7	p8
UFH 1/1 1 μl EC	66.1	8.3	11.7	2.6	3.4	1.6	0.6	5.6
UFH 1/2 1 μl EC	66.1	8.4	11.5	2.7	3.3	1.5	0.5	5.8
UFH 2/1 1 μl EC	66.0	8.5	11.8	2.8	3.4	1.8	0.4	5.5
UFH 2/2 1 μl EC	66.4	8.3	11.5	2.6	3.5	1.9	0.3	5.4
UFH 3/1 5 μl EC	65.7	8.3	11.4	2.7	3.6	2.0	0.4	5.9
UFH 3/2 5 μl EC	65.9	8.6	11.5	2.5	3.5	2.0	0.4	5.6

Table 2. Compositional analysis of UFH performed by CE can be used to rigorously compare different batches of LMWH. UFH was digested with either 1 μl or 5 μl of enzyme cocktail (EC). Each sample was digested in duplicate and each digest was analyzed in duplicate by CE. In all the samples, disaccharides p1-p7 had the same migration time. Comparison of duplicate analysis of the same sample (UFH 1/1 with UFH 1/2, UFH 2/1 with UFH 2/2, and UFH 3/1 with UFH 3/2) shows that there is good instrumental reproducibility. Comparison of either UFH 1/1 or UFH 1/2 with UFH 2/1 or UFH 2/2 shows that there is minimal run-to-run variation. Comparison of UFH digested with 1 μl of EC with UFH digested with 5 μl of EC illustrates that increasing the enzyme quantity does not change the disaccharide profile appreciably showing that exhaustive digestion is reached by using 1 μl of EC as shown in FIG. **1**.

Extension of CAM to LMWH Preparations. Given the ability of CAM to separate the enzymatically-derived components of UFH and to provide an accurate assessment of the overall composition of UFH, we sought to apply it to the structural analysis of LMWHs. Three different LMWHs were used, viz., tinzaparin, ardeparin, and enoxaparin, all of which are currently in clinical use (Table 3). Compositional com-

parison of the three LMWHs and UFH indicates that there are distinct differences in their structures, most notably in the mole % of the trisulfated disaccharide, p1, and the disulfated disaccharides p2 and p3, and the tetrasaccharide p8.

TABLE 3

Comparison of the disaccharide composition and anti-Xa activity of UFH and commercial LMWHs.

Saccharide	enoxaparin % of total	dalteparin % of total	UFH % of total
p1	63.6	62.1	66.1
p2	8.3	4.3	8.3
p3	11.3	9.8	11.6
p4	2.0	2.6	2.6
p5	3.5	1.4	3.4
p6	1.8	1.2	1.6
p7	1.9	5.4	0.6
p8	6.4	9.5	5.6
p9	0.5	0	0
p10	0.7	0	0
p11	0	3.7	0
Anti-Xa (IU/mg)	100	150	130
Anti-IIa (IU/mg)	25	60	130
MW (Da)	4,200	6,000	12,000

Peak 8 as an Indicator of Anticoagulant Function. To test whether quantification of 8 could be used to predict anticoagulant function, we plotted the anti-Xa or anti-IIa activity of UFH and LMWH's versus p8 content. Plot of Anti-IIa and Anti-Xa values of UFH, UFH size fractionated through Biogel P10 column, a LMWH generated in our laboratory, and commercial LMWHs demonstrates there is a linear correlation between the anti-Xa/IIa values, and the mole % of p8 of the preparation. Thus the anticoagulant and antithrombotic efficiency of heparin and LMWH can be estimated from their chemical composition. In the case of the anti-Xa activity, p8 content showed a very good correlation with activity (r^2=0.8) (FIG. **2**). An even better correlation (r^2=0.9) was observed when anti-IIa activity was plotted versus p8 content. Importantly, this correlation holds regardless of the source of the UFH or LMWH and the means by which the LMWH is generated. Thus, these results demonstrate that a particular structural motif, identified by CAM, , e.g., peak 8, can be used to predict both anti-Xa and anti-IIa activity.

Creation of a Second Generation LMWH: Based on the above findings, we examined whether it would be possible to create a LMWH with increased anti-Xa and anti-IIa activities in vitro. We reasoned that these activities could be increased by optimizing the p8 content of a LMWH preparation. To test this possibility, we digested UFH with heparinase under controlled conditions and monitored the p8 content as a result of enzymatic digestion. When the digestion was judged complete, the LMWH was purified by size fractionation, its MW assessed, and the anti-Xa and anti-IIa activities were determined. The in vitro profile of these new LMWHs were compared to that of enoxaparin, tinzaparin, and ardeparin (Table 3).

Notably, under two separate digestion and separation conditions, slightly different LMWHs were created. The first is hereafter referred to as second generation M118 and the latter as second generation M215. Molecular weight measurement of the two indicated that M118 possessed a molecular weight of 5,000 Da, while that of M215 was 4,500 Da (Table 4). Importantly, both were found to have a polydispersity of 1.0, that is, both of these LMWHs were less heterogenous than other LMWHs as well as UFH. In addition, as shown in Table 5, CAM analysis of the two indicated that they possess a higher weight percent of peak 8 than other LMWHs, thus we would predict that both of these compounds should have higher anti-Xa and anti-IIa activity than other LMWHs.

TABLE 4

Comparison of the biological activities of Mimeon's LMWH with other LMWH. MLMWHs have very high (>100 IU/mg) anti-IIa, and anti-Xa activities.

	Xa, IU/mg	IIa, IU/mg	Xa/IIa	IC_{50}, IIa	MW Da
M115	250	200	1.25	25.6	5000
M411	200	130	1.5	38.0	4500
enoxaparin	100	25	4.0	236	4200
ardeparin	93	60	1.5	98.3	5000

Table 5: Comparison of the Molecular Profile of Existing LMWH, and Heparin.

In vitro assessment of the activities of M115 and M411 indicated that, as predicted, M115 and M411 had higher anti-Xa activity. M115 had a measured anti-Xa activity of 330 IU/mg, over twice as high as UFH, and at least three times more than existing LMWHs. M411 was also a potent inhibitor of Xa, with an activity almost 1.5 times as high as UFH and approximately twice as great as existing LMWHs. Importantly, both M115 and M411 possessed significant anti-IIa activity of 200 IU/mg and 130 IU/mg, respectively. This is in contrast to existing LMWHs that exhibit 4-10 times less anti-IIa activity. These results are confirmed and extended by measuring the IC_{50} of these compounds for thrombin activation. Taken together, these results indicate that by designing a LMWH with higher p8 content, it is possible to create a LMWH with increased activity.

Example 2

M115 and M411 are Superior to other Heparins in both IIa and Xa Pharmacokinetics after s.c. Administration

M115 and M411 have markedly increased in vitro anti-Xa and Ia activity, expressed as IU per mg, compared to UFH or other LMWHs. A series of pharmacokinetic experiments using male New Zealand rabbits confirmed this in vivo. In these experiments, either UFH or LMWHs (M115 and M411) were administered to rabbits by subcutaneous injection. Then pharmacokinetic parameters were determined by following either the anti-Xa or IIa activities.

Methods

Male New Zealand rabbits weighing 2.5 to 3.0 kg were used for pharmacokinetics studies. After anesthesia with Ketamine (40 mg/kg) and Xyalazine (5 mg/kg), a 24-gauze Teflon catheter was inserted into to the center auricular artery. The catheter was connected to a heparin cap filled with isotonic saline. Heparin solutions were injected subcutaneously to the rabbits at 1 and 3 and 6 mg/kg. Four different heparins (UFH, Ardeparin, Enoxaparin, and F1) were included in this study. 0.2 ml of blood was withdrawn 0, 5, 10, 30 min, 1, 2, 3, 4, 6, 8, 10, 12, 14, 18, 24 hours after the injection. The first 0.2 ml blood withdrawn was discarded with each withdraw. Blood samples were collected in an aqueous solution of sodium citrate (3.8 mole %; 1/9, v/v), centrifuged at 2000×g for 20 min and the resulting plasma was shock frozen and stored in −80° C. freezer until assay.

All reagents (Coatest heparin kit, S2238 substrate, Thrombin) were purchased from Chromogenix (Diapharma Group,

Inc., OH). Anti-Xa assay was used to monitor plasma LMWH level. Anti-Xa assay was performed by modification of the amidolytic method of Teien and Lie (Thrombosis res. 10: 399-410, 1977) with Coatest heparin test kit by using S-2222 as the chromogenic substrate (Diapharma Group, Inc. OH). The detailed procedure was described elsewhere (Liu, etc., PNAS, 94: 1739-1744, 1997). The concentration of LMWH in unknown samples was calculated by comparing to the calibration curve derived from 1st international standard for LMWH which was linear in the range of 0-0.7 IU/ml (r^2>0.99). The results were expressed in anti-Xa IU/mg and then in μg/ml. Anti-IIa assay was done similarly by using S2238 as substrate. Both Xa and Ia assays were performed by an automated coagulation machine (Coag-A-Mate MTX II, Organon Teknika Durham, N.C. 27712).

Results

At an equivalent dose of 3 mg/kg, the pharmacokinetic parameters derived from following the anti-Xa activity present in the plasma demonstrated that the bioavailability of M118 is about 3-4 fold higher than either UFH or other LMWHs (FIG. **3**). M115 exhibits comparable absorption (k_a) and elimination (k_e) rate constants (Table 6) compared to UFH, demonstrating that the increased bioavailability is due to the higher inherent anti-Xa activity (IU/mg) of M115 as compared to other heparins (data not shown). This observation is consistent with the in vitro activities of M115. Thus, the absorption and elimination of M115 is as efficient as other heparins. As a result, a much higher plasma anti-Xa activity is achieved when the same dose is administered to the animals.

TABLE 6

The plasma anti-Xa pharmacokinetics parameters after s.c. administration.

	UFH	M115	ENOXAPARIN	DALTEPARIN
Ka	0.25	0.16	0.43	0.45
Ke	0.16	0.12	0.31	0.23
$t_{1/2a}$(hr)	2.85	5.27	1.67	1.73
$t_{1/2e}$(hr)	4.65	8.20	2.25	3.41
AUC(IU*hr/ml)	7.97	34.24	7.26	9.97
C_{max}(IU/ml)	0.51	3.53	1.20	2.00
t_{max}(hr)	5.16	11.87	2.76	3.20
MRT(AUMC/AUC)	6.22	9.96	5.01	5.73

To test whether the plasma anti-IIa activity can be correlated to the anti-Xa pharmacokinetics, plasma anti-IIa pharmacokinetics for UFH and the LMWHs was also established. Consistent with the observed difference in in vitro anti-IIa activity, the plasma anti-IIa pharmacokinetics result showed much higher bioavailability for M115 and M411 as compared to other heparins. This is especially true when one compares either enoxaparin or UFH with either M115 and M411. For enoxaparin, the significant observed difference can be attributed to the fact that enoxaparin possesses inherently low anti-IIa activity (~25 IU/mg compared to ~250 IU/mg for M115). In the case of UFH, its increased polydispersity results in the administration of some larger polysaccharide fragments that are eliminated faster, reducing bioavailability.

Example 3

M115 and M411 are a More Potent Inhibitor of Arterial Thrombosis

The formation of arterial thromboses is largely due to the activation and aggregation of platelets. Activated thrombin (IIa) is known to be a potent activator of platelet aggregation, hence, molecules containing high anti-IIa activity should be more potent inhibitors of arterial thrombosis formation. We investigated whether or not M115 and M411 produced a more pronounced antithrombotic effect using a rat arterial thrombosis model.

Methods

The arterial thrombosis model was performed essentially as described with minor modification. Male Sprague-Dawley rats weighing 350-400 g were anesthetized with Ketamine (80 mg/kg) and Xylazine (10 mg/kg). The right side carotid artery was carefully isolated free of surrounding tissues (about 2 cm). A perivascular probe connected to an ultrasonic flow meter (Transonic Flow Meter, NY) was placed under and surrounding the carotid artery to monitor the blood flow rate. The experiments began with the injection of 0.2 ml of either saline or heparin solution via the penile vein. Exactly 1 min after injection, a piece of filter paper (6 mm in diameter, Whatman #5) soaked with 50 mole % $FeCl_3$ was placed on top of freed carotid artery. The filter paper was removed 15 minutes later. The experiment was terminated 1 h after $FeCl_3$ treatment and the carotid artery (2 cm) was removed. The thrombus (if formed) was removed and weighed wet. The total occlusion time (TOT), the time it takes for the blood flow to completely stop, as well as the thrombus weight were recorded.

Results

FIG. **4** shows the anti thrombotic activity of heparin in the rat arterial thrombosis model as well as the thrombus weight. Thrombus was weighed at the end of the 1 hour thrombus induction period. The total occlusion time and thrombus weight as a function of different heparins at different doses is given in FIG. **4**. At 0.5 mg/kg, UFH prolonged the total occlusion time (TOT) to about 27 minutes compared to that of 17 minutes for the control group. It is noticed that a much lower dose is required for F1 to achieve a similar antithrombotic effect as that of UFH and Enoxaparin. A slightly weaker inhibition was observed for enoxaparin (TOT=23 min). This inhibition of thrombus formation was also observed in the final thrombus weight.

In contrast, at the same dose of 0.5 mg/kg, M115 completely prevented the occlusion of the artery. In this case, the blood flow rate never reached 0 within the 60 minutes observation window. This is also reflected by the significantly reduced thrombus weight at the end of 60 minutes. At 0.3 mg/kg, essentially the same responses were observed, namely no complete occlusion occurred and a significantly reduced thrombus weight was observed within the 60 minute period. At 0.1 mg/kg, the TOT and thrombus weight of M115 treated group became comparable to that observed for UFH and enoxaparin at 0.5 mg/kg. M411 was also an extremely potent inhibitor of arterial thrombosis formation, though less so than M115, as expected from its slightly decreased anti-IIa activity in vitro. Thus, a higher anti-IIa activity is associated with more potent inhibition of arterial thrombosis formation. In addition, the increased potency of M115 and M411 is consistent with their in vitro activity as well their favorable pharmacokinetics, especially bioavailability.

Example 4

s.c. Administered M115 and M411 is Associated with Increased Plasma TFPI Activity

Accumulating evidence indicates that the complex tissue factor (TF)- activated factor VIIa (FVIIa) is a key initiator of arterial thrombosis in vivo. TFPI is a potent inhibitor of the

tissue factor coagulation pathway, which exerts its function by neutralizing the catalytic activity of factor Xa and by feedback inhibition of the factor VIIa-TF complex in the presence of factor Xa. UFH and LMWH, in addition to their well-studied ability to promote the inhibitory activity of ATIII, also release TFPI from endothelial cells. This function further contributes, in a dramatic fashion, to the overall anti-coagulant and antithrombotic activity of heparin and LMWHs. In fact, studies have found that LMWHs are known to more efficiently release TFPI into the blood and thereby promote a favorable anticoagulant function as compared to UFH. Given the importance of TFPI release in the overall function of pharmacologic UFH and LMWHs, we sought to measure the effect of M115 and M411 on TFPI release in vivo. We measured the activity of TFPI in the plasma after s.c. administration of M115 and M411, UFH, or dalteparin as a model LMWH. To establish a release profile, plasma samples collected at different time points were tested.

Methods

TFPI activity in rabbit plasma after single s.c. administration of heparin was determined by a 2-step colorimetric assay. Briefly, in the first step, a dilution of the test sample was incubated with a saturating concentration of FVII/IIa complex. In the second step, a high concentration of FX was added to the reaction mixture as a substrate for the residual FVIIa-TF catalytic activity; the FXa generated is measured with a specific chromogenic substrate (American Diagnostica Inc, Connecticut). The absorbance was read at 405 nm. Linear calibration curves were obtained with standard plasmas provided by the manufacture (American Diagnostica Inc). All test samples were assayed at a 5 mole % dilution. Results are expressed as percent of TFPI activity in pooled rabbit plasma.

Results

TFPI release profiles after s.c. administration of different heparins at 3 mg/kg are shown in FIG. **5**. The release of TFPI is reflected by percentage increase in the plasma TFPI activity as determined by a chromogenic assay. It is noticed that Fl treatment led to a significant higher level of TFPI activity, which also persisted longer than other heparin treatments.

Compared to UFH and dalteparin, s.c. administration of either M115 or M411 is associated with a more pronounced release of TFPI into the circulation. The peak TFPI activity is reached about 4 hours after s.c. administration. TFPI activity is also elevated in the plasma from UFH treated animals, albeit, to a lesser extent. Surprisingly, dalteparin, a LMWH, only resulted in minimal increase of plasma TFPI activity. The results from this experiment strongly suggest that the administration of M115 or M411 is associated with superior mobilization of TFPI from the endothelium, more so than either UFH or dalteparin.

Example 5

M115 or M411 are more Potent Anticoagulants than UFH

Anti-coagulation has been the primary clinical application for UFH for over 65 years. Due to its erratic pharmacokinetics following s.c. administration, UFH has been administered by intravenous injection instead. Additionally, the application of UFH as an anticoagulant has been hampered by the many side effects associated with non-specific plasma protein binding with UFH. Therefore, it is important to develop a novel LMWH that retains the anticoagulant activity of UFH but has reduced side effects. LMWHs, essentially due to their reduced chains sizes and dispersity, display markedly less non-specific plasma protein binding. However, all LMWHs that are currently clinically available also possess reduced anti-IIa activity compared to UFH. Because of this decreased activity, a larger dose of LMWH is required (compared to UFH) in order to achieve a similar anti-coagulant activity. Consequently, the use of LMWHs so far has been largely limited to the prevention of thrombosis and not to their treatment.

Methods

The second generation LMWHs reported here are unique for a number of reasons. First, while M115 and M411 have lower molecular weight than UFH and are in the accepted molecular weight range for LMWHs, these molecules possess high anti-Xa and IIa activities, 2-4 times higher than that of UFH or other LMWH on a mass basis. In addition, when compared to a typical LMWH, both M115 and M411 have 5-10 times higher anti-IIa activity as well as enriched anti-Xa activity. The efficiency of M115 and M411 as anticoagulants was compared to that of conventional UFH. To test this, a rat tail bleeding time assay was completed. The bleeding time was determined with a rat model as described with minor modifications. Specifically, male Sprague-Dawley rats weighing 350-400 g were used. Intraperitoneal injection of Pentobarbital at 55 mg/kg was used for anesthesia. Saline or heparin solution were injected via the penile vein of the rats. 1 min after injection, rat tail was cut 2 mm from the tip with a razor blade. The bleeding tail was blotted with a Whatman #3 filter paper every 30 seconds until the blot is free of blood, and the time was recorded.

Results

Both M115 or M411 showed a much more potent anticoagulant effect in this model, consistent with their increased anti-Xa and IIa activity. At 0.5 mg/kg, the bleeding time of sgL-1 treated rats exceeded 60 min compared to that of 20 minutes for UFH. At 0.3 mg/kg, the bleeding time became comparable to that of UFH and at 0.1 mg/kg the bleeding time returned to baseline level. Similarly, rats treated with M411 demonstrated markedly longer bleeding times than those treated with UFH.

Example 6

Creation of a Panel of LMWH with Different Ratios of anti-Xa and anti-IIa Activity

One of the drawbacks associated with the LMWH therapies currently known in the art is an inability to individually tailor LMWH treatment to a subject. Until now, there has not been a preparation that is both sufficiently well characterized and consistent from batch to batch, as the methods for preparing LMWHs known in the art were inadequate to produce such preparations. The methods of the invention allow the preparation of consistent and predictable compositions of LMWH with desired properties, for instance, a LMWH preparation with a given ratio of anti-Xa:anti-IIa activity. This method can be used to produce a panel of LMWH preparations with varying degrees of anti-IIa and anti-Xa activity, among other characteristics. This method is not limited to manipulating anti-IIa or anti-Xa activity, but can be extended by using the methods disclosed and claimed herein to produce LMWH preparations with other desired characteristics, such as ultra-low molecular weight, PF4 binding, protamine neutralization, FGF binding, etc. The compositions made by this method can then be used to tailor treatment if a subject to their status; for instance, in the treatment of a clot, it might be advantageous to administer a LMWH preparation having

high anti-Xa/anti-IIa activity early in the treatment cycle, and later switch to a LMWH preparation having only anti-Xa activity.

Methods.

A "grid" procedure was used to make a number of LMWH preparations with variable structural signatures. One example, not meant to be limiting, of a grid is illustrated below; it is used by moving down the grid from top to bottom, choosing any one of the options available in each row. Each option is intended as a guide and one of ordinary skill in the art will understand that options between and beyond those illustrated below are within the scope of the invention. Specific examples using the grid are described below. As one example, one may start with UFH, at a concentration of 10 mg/ml, precipitate with $MgCl_2$, choose methanol for use as the polar solvent for steps 1 and 2, and so on and so forth. It is not necessary to stay in a single column; the choice of an option may affect the structural signature of the resulting composition.

Starting material	Un-fractionated Heparin	enoxaparin	dalteparin	Other LMWH
Concentration of Starting material	1 mg/ml	10 mg/ml	100 mg/ml	1 g/ml
Salt Type	NaCl	Na-acetate	$MgCl_2$	Other salt
Polar solvent used in step 1, and 2	Acetone	Ethanol	Methanol	Other solvent
Quantity of Polar solvent used	0.1 V (where 1 V = volume of heparin solution in water)	1 V	2 V	10 V
Reaction Time for step 1	1 h	6 h	12 h	24 h
Reaction Time for step 2	1 h	6 h	12 h	24 h
Reaction Time for step 3	1 h	6 h	12 h	24 h
Reaction Temperature for step 1	0 C.	4 C.	10 C.	RT
Reaction Temperature for step 2	0 C.	4 C.	10 C.	RT
Reaction Temperature for step 3	10 C.	RT	37 C.	45 C.
Depolymerizing agent (Enzyme/ Chemical/ Energy source like γ-radiation)	Heparinase 1	Heparinase II	Heparinase III	Heparinase IV or mammalian Heparanase

Synthesis of Enoxaparin-derived LMWH Compounds:

Step 1: 100 mg of enoxaparin was dissolved in 10 ml of water to get 10 mg/ml concentration. 100 mg NaCl was added to this solution. The pH of the solution was adjusted to 6.7. 5 ml 200 Proof ethanol was added to this mixture. The solution was maintained at 4 C for 24 h. The residue (MLP) that is precipitated is removed by centrifugation at 4000 RPM for 15 min. 20 ml ethanol was added to the supernatant, and the mixture maintained at 4° C. for 24 h. The precipitate formed at the end of 24 hours (MLS) is separated by centrifugation at 4000 RPM for 15 min. It is lyophilized overnight to give 60 mg dry powder of MLS.

Step 2: 100 mg MLS was dissolved in 10 ml of 50 mM Calcium Acetate buffer, pH 6.7. An enzyme cocktail consisting of 10 mg Heparinase II and 1 mg of Heparinase III was added to this mixture, and the solution was maintained at 37° C. for 4 h. The precipitate formed at the end of 2 hours was removed by centrifugation at 4000 RPM for 15 min. The supernatant of digested MLS was desalted in a size exclusion chromatography column.

Step 3: 100 mg MLS digested by the method explained above was loaded on a 1 m long, 10 cm diameter P10 size exclusion column. 500 mM Ammonium Acetate buffer was used as the running buffer. The eluent was tracked by absorption at UV 232 nM. 3 ml peaks were collected after the initial void volume. The peaks that gave absorption of more than 0.1 unit were collected. They were divided into 10 equal fractions. The different fractions were then lyophilized from water to get rid of ammonium bicarbonate salt. They were then assayed for the building blocks and functional characteristics (anti-Xa, and anti-IIa activity) by the assays described. Characteristics of Fraction 3 and Fraction 7 (named as M108, and M405) are listed in the table below.

Synthesis of UFH-derived LMWH Compounds:

Step 1: 100 mg of UFH was dissolved in 10 ml of water to get 10 mg/ml concentration. 100 mg NaCi was added to this solution. The pH of the solution was adjusted to 6.7. 3 ml 200 Proof ethanol was added to this mixture. The solution was maintained at 4 C for 12 h. The residue (MUP) that is precipitated is removed by centrifugation at 4000 RPM for 15 min. 10 ml ethanol was added to the supernatant, and the mixture maintained at 4° C. for 24 h. The precipitate formed at the end of 24 hours (MUS) is separated by centrifugation at 4000 RPM for 15 min. It is lyophilized overnight to give 60 mg dry powder of MUS.

Step 2: 100 mg MUS was dissolved in 10 ml of 50 mM Calcium Acetate buffer, pH 6.7. An enzyme cocktail consisting of 5 mg Heparinase II and 5 mg of Heparinase III was added to this mixture, and the solution was maintained at 37° C. for 4 h. The precipitate formed at the end of 2 hours was removed by centrifugation at 4000 RPM for 15 min. The supernatant of digested MUS was desalted in a size exclusion chromatography column.

Step 3: 100 mg MUS digested by the method explained above was loaded on a 1 m long, 10 cm diameter P10 size exclusion column. 500 nM Ammonium Acetate buffer was used as the running buffer. The eluent was tracked by absorption at UV 232 nM. 3 ml peaks were collected after the initial void volume. The peaks that gave absorption of more than 0.1 unit were collected. They were divided into 10 equal fractions. The different fractions were then lyophilized from water to get rid of ammonium bicarbonate salt. They were then assayed for the building blocks and functional characteristics (anti-Xa, and anti-IIa activity) by the assays described. Characteristics of Fraction 2 and Fraction 4 (designated M115, and M411) are listed below.

Results.

The methods described above were used to prepare and characterize the following LMWH compositions:

TABLE 7

Novel LMWH compositions, AUC as determined by CE analysis.

AUC %	M108	M405	M115	M411
p1	60.9	61.9	53.8	54.0
p2	6.8	8	5.7	6.6
p3	14.7	10.4	18.5	18.7
p4	2.7	1.6	3.4	3.5
p5	1.6	4.3	0.4	0.5
p6	2.3	3.9	1.4	1.6
p7	4.4	5.8	9	8.9
p8	6.1	2.6	8.1	6.2
p9	0.3	0.7		
p10	0.2	0.6		
Anti-Xa, IU/mg	150	80	250	200
Anti-IIa IU/mg	130	0	200	130
MW, Da	5000	2200	5000	4500

TABLE 8

Novel LMWH compositions, mole % of given components.

Mole %	M108	M405	M115	M411
p1	62.7	67.0	55.3	56.7
p2	6.3	7.7	5.2	6.2
p3	12.6	9.3	15.8	16.3
p4	2.3	1.4	2.9	3.0
p5	1.0	2.8	0.2	0.3
p6	1.0	1.7	0.6	0.7
p7	2.6	3.6	5.3	5.3
p8	11.1	5.0	14.7	11.5
p9	0.3	0.8	250	200
p10	0.2	0.6		
Anti-Xa, IU/mg	150	80	250	200
Anti-IIa IU/mg	130	0	200	130
MW. Da	5,000	2200	5000	4500

We used the "grid" procedure described above for making M108, M405, M115, and M411, the specific examples mentioned above. It is to be understood that these are complex molecules obtained from a complex starting material by varying multiple parameters. Since the composition of the product is affected by multiple parameters, adjusting different parameters in different ways, and monitoring the profile of the product, would allow one of ordinary skill in the art to prepare products similar to M108, M405, M115, and M411.

The parameters that can be varied include, but are not limited to:

1) Starting material: UFH, FH, other LMWH preparations such as enoxaparin (Lovenox™); dalteparin (Fragmin™); certoparin (Sandobarin™); ardeparin (Normiflo™); nadroparin (Fraxiparin™); parnaparin (Fluxum™); reviparin (Clivarin™); tinzaparin (Innohep™ or Logiparin™), among others.
2) Salt (type, concentration): such as divalent metals such as Mg, and Ca (e.g., $MgCl_2$, Calcium acetate, etc.).
2) Enzyme (Heparinase I, II, III, IV, heparanases, mutant heparinases, and different combinations of these enzymes).
3) Temperature
4) Incubation time

This method has been used to create LMWH preparations with different characteristics. For instance, LMWH preparations which are fully neutralized by protamine can be created, such that the addition of protamine neutralizes anti-Xa activity by ≧50% and anti-IIa activity by ≧70%. As can be seen in FIGS. **6**, **7** and **8**, novel LMWH preparations M118 and M312 (which are prepared in a manner similar to M115, and M411) are both more sensitive to protamine neutralization of anti-Xa and anti-IIa activity than either UFH or enoxaparin. In addition, LMWH preparations with lower PF4 binding activity have been created, as can be seen in table 9, these preparations have lower amounts of components 1, 2, 4, and 6, which are associated with PF4 binding; see also FIG. **9**. Since PF4 binding has been linked to heparin induced thrombocytopenia (HIT), a composition of LMWH with decreased PF4 binding would be very desirable.

PF4 binding was assayed using the filter binding assay of Maccarana et al. Briefly, 1 μg of 3H-radiolabeled heparin is incubated with 1 μg of PF4 in the presence of various amounts of nonradioactive LMWHs for 10 min at 37° C. in 10 μl of Tris buffer (130 mM NaCl, 50 mM Tris-HCl, pH 7.3). The volume is then made up to 300 μl by the addition of Tris buffer, and the samples are drawn through buffer-equilibrated cellulose nitrate filters on a vacuum manifold. The filters are washed with 2×5 ml of 130 mM NaCl, 50 mM Tris-HCl, and bound material eluted with 2×5 ml of 2 M NaCI, 50 mM Tris-HCl. On average greater than 99% of the radiolabeled material was removed from the filters with 2 M NaCl, 50 mM Tris-HCl.

To assess PF4 binding affinity for the various LMWHs, Scatchard analysis of the data collected by the filter binding assay was used. The lines of best fit and graphical equations for the data were determined. The gradients of these lines are equivalent to 1/Kd(1) and 1/Kd(2), the x intercept for the first line represents the number of binding sites on the protein (n1), and the x intercept for the second represents n1+n2, where n2 is the number of binding sites with Kd(2).

TABLE 9

Comparison of equivalent Anti-Xa activity for side effects

Saccharide components	Enoxaparin	M118	M312
Total (mg)	100	32.0	48.4
p1 (mg)	63.5	18.9	29.7
p2 (mg)	7.2	1.8	3.3
p4 (mg)	2.1	0.4	0.9
p6 (mg)	2.0	0.1	0.3
Anti-Xa (IU)	100	100	100
MW (Da)	4,200	5,000	4,500

As is apparent from these results, the methods can be used to create a LMWH preparation with almost any characteristic desired, including varying ratios and levels of anti-Xa and anti-IIa activity; protamine neutralization; FGF binding; and PF4 binding.

Example 7

LMWH Preparations with Low Batch-batch Variability

One of the great drawbacks of the UFH and LMWH preparations currently known in the art is their great variability in both composition and in activity. This has limited the population of patients for whom LMWH or UFH therapy was indicated, for instance excluding patients with abnormal renal function, among others. Abnormal renal function is measured by urea, creatinine, phosphorus, GFR or BUN in blood and urine. Administration of the known LMWH preparations is often a trial and error approach of titrating the appropriate dosage based on inaccurate tests, which can lead to unwanted and severe side effects such as post-operative bleeding. It would be greatly desirable to have a method for making

LMWH preparations with low batch-batch variability and a desired structural signature. The methods of this invention allow for the creation of such preparations.

Methods.

Several enoxaparin preparations were depolymerized by a cocktail of enzymes, including heparinases. Next, a capillary electrophoresis (CE) profile of the resulting digest was run in an Agilent CE instrument in the negative mode. Shown in the table below are the disaccharide building blocks seen in three batches of commercially available enoxaparin. The composition is expressed as mole % of the building blocks of enoxaparin. This table teaches the composition as a mole % of the constituent building blocks. In, other words, one mole of enoxaparin is composed of X1 mole % of disaccharide building block 1, X2 mole % of disaccharide building block 2, . . . , XN mole % of building block "N". X1+X2+ . . . +Xn=100. The variation was calculated by taking the average of the three values, and dividing the largest deviation by the average.

TABLE 10

Enoxaparin Batch-to-batch Variation, mole %.

Saccharide	Enox. Batch 1	Enox. Batch 2	Enox. Batch 3	Variation (%)
p1	60.8	63.5	63.6	4
p2	7.0	7.2	8.3	17
p3	11.8	10.8	11.3	9
p4	2.5	2.1	2.0	23
p5	3.6	3.5	3.5	3
p6	1.8	2.0	1.8	11
p7	5.4	4.3	1.9	91
p8	6.6	5.8	6.4	13
p9	0.2	0.4	0.5	82
p10	0.3	0.4	0.7	86

The table above demonstrates that the variation between batches of commercially available enoxaparin (Lovenox™) is substantial. To alleviate this problem, the methods of the current invention alleviate this problem by providing a method for quality control.

Results.

One example, not meant to be limiting, of the application of this method is as follows. First, a desired reference structural signature, mole %, or activity is selected, based upon a standard preparation that has, for instance, the desired activity at desired levels. Using the data in table 10, and maximizing for anti-Xa activity, a range of acceptable values would be chosen for mole % of peak **8**, for example, 6.5 mole %. Within the scope of the invention, each batch of enoxaparin that is manufactured would then be subjected to the analysis methods of the invention, to determine the mole % of 8. Batches of enoxaparin that fell within a given variation of the desired range would be accepted; those that did not would be rejected. Again taking the data from table 10 for an example, if the desired mole % is 6.5, and the acceptable variation is 5%, then only those batches with a mole % of the peak 8 tetrasaccharide of 6.5±0.3 would be accepted. Thus, Batches 1 and 3 would be acceptable, but Batch 2 would be rejected as having insufficient levels of p8 (and thus insufficient levels of anti-Xa activity).

Further applications of this method include determining the structural signature of the starting material, e.g. porcine intestinal mucosa heparin. This starting material is isolated in slaughter houses and is often unmonitored by standard quality control techniques. Using the methods described above to ensure that the quality of the starting material, e.g., the structural signature and activity, is sufficient to produce acceptable LMWH preparations. Adding this quality control to the beginning of the procedure so that the starting material is consistent helps to decrease batch-batch variability, and thus decrease the number of rejected batches, saving time and money, and resulting in an improved product.

Example 8

Monitoring a Subject

The ability to track and monitor LMWH preparations in a subject, such as a human or veterinary subject, or an experimental animal, would greatly enhance both research and therapeutic applications of these preparations. To date, monitoring methods have relied on activity assays that suffered from numerous drawbacks, as described above.

Methods

Following administration of a LMWH preparation to a subject, e.g., a human or veterinary subject, or an experimental animal, a sample or samples are taken from that subject at various periods of time. The sample can be any bodily fluid, including but not limited to blood or urine. The sample is then purified by appropriate methods known in the art, such as those disclosed in U.S. Pat. No. 5,843,786; the method of purification will depend on the sample type. As one example, not meant to be limiting, the sample is blood. After removal of the whole cells by filtration or centrifugation, further filtration may be utilized to rid the sample of high molecular weight contaminants. The sample may be further purified to remove neutral contaminants by ion exchange methods conventionally known in the art. The sample may then be derivatized using methods known in the art. Finally, the sample is treated using the methods described above to depolymerized the polysaccharides prior to analysis, e.g., by CE, MALDI-MS, and/or PEN-MALDI. The sample may also be compared to a reference to quantify the levels of LMWH in the sample.

As one example, not meant to be limiting, the method is as follows. After s.c. or i.v. injection of heparin or LMWH, blood or urine samples were collected at selected timepoints. Samples were purified bound to a micro-DEAE column (Pharmacia-Biotech), washed with a buffer of 10 mM phosphate, 0.1M NaCl pH 6.0 and eluted with 10 mM phosphate 1M NaCl pH 6.0. The sample was then further purified and concentrated on a Microcon-3 spin column prior to enzymatic digestion and compositional analysis.

The sample was then subjected to exhaustive depolymerization with an enzyme cocktail made up of heparinase I, II, and heparinase III. 9 μl of 10 μg/μl concentration of UFH in H_2O was digested with 1 μl of an enzyme cocktail consisting of 100 nM each of heparinase I, II, and III in 25 mM sodium acetate, 100 mM sodium chloride, 5 mM calcium acetate buffer, pH 7.0 for 12 hours at 37° C. The CE sample was prepared by diluting 1 μl of the digest with 9 μl of H_2O. The samples were analyzed by CE in reverse polarity with a running buffer of 50 mM tris/phosphate, 10 μM dextran sulfate, pH 2.5. The results are shown in FIG. **10**. Using this method, the LMWH preparations can be monitored over time in a subject; the results are plotted against time, as is shown in FIGS. **11** and **12**.

Example 9

Tagged LMWH Preparations

The ability to track and monitor LMWH preparations in a subject, such as a human or veterinary subject, or an experi-

mental animal, would greatly enhance both research and therapeutic applications of these preparations. The use of a marker or tag built into the LMWH preparation significantly eases monitoring, quantitation and detection.

Methods.

Following the preparation of a LMWH, either by the methods disclosed herein or other methods known in the art, a label is attached to one or more of the constituent of the LMWH. Such a label can be a fluorophore (Morell et al., *Electrophoresis* (1998) 19(15):2603-11; Anumula et al., *Glycobiology* (1998) 8(7):685-94; Sudor et al., *Anal Chem* (1997) 69(16): 3199-204; Bigge et al., *Anal Biochem* (1995) 230(2):229-38; Franz et al., *J Am Soc Mass Spectrom* (2001) 12(12):1254-61; Drummond et al., *Proteomics* (2001) 1(2):304-10; Araki et al., *J Chromatogr B Biomed Sci* (2001) 753(2):209-15; Li et al., *Anal Biochem* (1993) 211(2):250-7); biotin (Imai et al., *FEBS Lett* (2002) 510(3):201-5; radioactive isotopes (Collard et al., *Anal Biochem* (1997) 247(2):448-50); mass-label; antigenic moieties, or other suitable labels known in the art. Preferably, the label is attached to an active constituent of the LMWH.

Thus labeled, the LMWH can be detected and quantified by methods known in the art. As one example, not meant to be limiting, a human or veterinary subject, or an experimental animal, is treated with a LMWH preparation including a tag. Then, a sample is taken from that subject. The sample may be subjected to purification under appropriate conditions known in the art, such as those disclosed in U.S. Pat. No. 5,843,786. The tag is then detected using appropriate methodology known in the art; for instance, if a fluorescent tag is incorporated into the LMWH preparation, fluorescence detection procedures may be utilized, such as is described in Araki et al., *J Chromatogr B Biomed Sci* (2001) 753(2):209-15.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The invention is not to be limited in scope by examples provided, since the examples are intended as a single illustration of one aspect of the invention and other functionally equivalent embodiments are within the scope of the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims. The advantages and objects of the invention are not necessarily encompassed by each embodiment of the invention.

All references, patents and patent publications that are recited in this application are incorporated in their entirety herein by reference.

We claim:

1. A method for analyzing an enoxaparin sample for the presence or amount of a non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of making enoxaparin that included β-eliminative cleavage with a benzyl ester and depolymerization, comprising:

providing an enoxaparin sample that has been exhaustively digested with two or more heparin degrading enzymes;

using a separation method to determine, in the enoxaparin sample that has been contacted with two or more heparin degrading enzymes, the presence of a structural signature associated with the non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of making enoxaparin that includes β-eliminative cleavage with a benzyl ester and depolymerization;

making a determination about the enoxaparin sample based upon a comparison of the determination of the presence of a structural signature associated with the non naturally occurring sugar associated with peak 9 to a reference standard for enoxaparin, and

determining the presence of the structural signature associated with the non naturally occurring sugar associated with peak 9 of FIG. **1** in a second batch of enoxaparin,

to thereby analyze the enoxaparin sample.

2. The method of claim **1**, further comprising selecting a batch as a result of the determination based upon comparison to the reference standard.

3. A method for analyzing an enoxaparin sample for the presence or amount of a non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of making enoxaparin that included β-eliminative cleavage with a benzyl ester and depolymerization, comprising:

providing an enoxaparin sample that has been exhaustively digested with two or more heparin degrading enzymes;

using a separation method to determine, in the enoxaparin sample that has been contacted with two or more heparin degrading enzymes, the presence of a structural signature associated with the non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of making enoxaparin that includes β-eliminative cleavage with a benzyl ester and depolymerization; and

making a determination about the enoxaparin sample based upon a comparison of the determination of the presence of a structural signature associated with the non naturally occurring sugar associated with peak 9 to a reference standard for enoxaparin, wherein the determination made regarding the comparison to the reference standard is whether to keep or discard the sample,

to thereby analyze the enoxaparin sample.

4. The method of claim **3**, wherein the sample is kept.

5. The method of claim **3**, wherein the sample is discarded.

6. A method for analyzing an enoxaparin sample for the presence or amount of a non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of making enoxaparin that included β-eliminative cleavage with a benzyl ester and depolymerization, comprising:

providing an enoxaparin sample that has been exhaustively digested with two or more heparin degrading enzymes;

using a separation method to determine, in the enoxaparin sample that has been contacted with two or more heparin degrading enzymes, the presence of a structural signature associated with the non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of making enoxaparin that includes β-eliminative cleavage with a benzyl ester and depolymerization; and

making a determination about the enoxaparin sample based upon a comparison of the determination of the presence of a structural signature associated with the non naturally occurring sugar associated with peak 9 to a reference standard for enoxaparin, wherein the determination based upon the comparison to the reference standard regards the quality of the sample,

to thereby analyze the enoxaparin sample.

7. A method for analyzing an enoxaparin sample for the presence or amount of a non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of making enoxaparin that included β-eliminative cleavage with a benzyl ester and depolymerization, comprising:

providing an enoxaparin sample that has been exhaustively digested with two or more heparin degrading enzymes;

using a separation method to determine, in the enoxaparin sample that has been contacted with two or more heparin degrading enzymes, the presence of a structural signa-

ture associated with the non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of making enoxaparin that includes β-eliminative cleavage with a benzyl ester and depolymerization; and

making a determination about the enoxaparin sample based upon a comparison of the determination of the presence of a structural signature associated with the non naturally occurring sugar associated with peak 9 to a reference standard for enoxaparin, wherein the determination based upon the comparison to the reference standard regards bioequivalence of the sample,

to thereby analyze the enoxaparin sample.

8. A method for analyzing an enoxaparin sample for the presence or amount of a non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of making enoxaparin that included β-eliminative cleavage with a benzyl ester and depolymerization, comprising:

providing an enoxaparin sample that has been exhaustively digested with two or more heparin degrading enzymes;

using a separation method to determine, in the enoxaparin sample that has been contacted with two or more heparin degrading enzymes, the presence of a structural signature associated with the non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of making enoxaparin that includes β-eliminative cleavage with a benzyl ester and depolymerization;

making a determination about the enoxaparin sample based upon a comparison of the determination of the presence of a structural signature associated with the non naturally occurring sugar associated with peak 9 to a reference standard for enoxaparin;

detecting one or more of anti-Xa activity, anti-IIa activity, molecular weight distribution and average molecular weight of the sample; and

comparing any of anti-Xa activity, anti-IIa activity, molecular weight distribution and average molecular weight of the sample to a reference standard for enoxaparin,

wherein the reference standard for molecular weight distribution is less than or equal to 20% are <2000 Da species, greater than or equal to 68% are 2000-8000 Da species, and less than or equal to 18% are >8000 Da species,

to thereby analyze the enoxaparin sample.

9. The method of claim **8**, wherein the reference standard for anti-Xa activity is 100 IU/mg.

10. A method for analyzing an enoxaparin sample for the presence or amount of a non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of making enoxaparin that included β-eliminative cleavage with a benzyl ester and depolymerization, comprising:

providing an enoxaparin sample that has been exhaustively digested with two or more heparin degrading enzymes;

using a separation method to determine, in the enoxaparin sample that has been contacted with two or more heparin degrading enzymes, the presence of a structural signature associated with the non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of making enoxaparin that includes β-eliminative cleavage with a benzyl ester and depolymerization;

making a determination about the enoxaparin sample based upon a comparison of the determination of the presence of a structural signature associated with the non naturally occurring sugar associated with peak 9 to a reference standard for enoxaparin;

detecting one or more of anti-Xa activity, anti-IIa activity, molecular weight distribution and average molecular weight of the sample; and

comparing any of anti-Xa activity, anti-IIa activity, molecular weight distribution and average molecular weight of the sample to a reference standard for enoxaparin, wherein the reference standard for average molecular weight is 4500 Da,

to thereby analyze the enoxaparin sample.

11. A method for analyzing an enoxaparin sample for the presence or amount of a non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of making enoxaparin that included β-eliminative cleavage with a benzyl ester and depolymerization, comprising:

providing an enoxaparin sample that has been exhaustively digested with two or more heparin degrading enzymes;

using a separation method to determine, in the enoxaparin sample that has been contacted with two or more heparin degrading enzymes, the presence of a structural signature associated with the non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of making enoxaparin that includes β-eliminative cleavage with a benzyl ester and depolymerization; and

making a determination about the enoxaparin sample based upon a comparison of the determination of the presence of a structural signature associated with the non naturally occurring sugar associated with peak 9 to a reference standard for enoxaparin,

wherein the method comprises determining the presence of the structural signature associated with the non naturally occurring sugar associated with peak 9 of FIG. **1** that results from the method used to make enoxaparin and at least one other structural signature,

wherein at least 32 structural signatures are determined,

to thereby analyze the enoxaparin sample.

12. The method of claim **11**, wherein the other structural signature is associated with one or more components of peaks 1, 2, 3, 4, 5, 6, 7 and 8 of FIG. **1**.

13. The method of claim **11**, wherein the presence of one or more structural signatures of components associated with peak 1 or peak 8 of FIG. **1** is determined.

14. A method for analyzing an enoxaparin sample for the presence or amount of a non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of making enoxaparin that included β-eliminative cleavage with a benzyl ester and depolymerization, comprising:

providing an enoxaparin sample that has been exhaustively digested with two or more heparin degrading enzymes;

using a separation method to determine, in the enoxaparin sample that has been contacted with two or more heparin degrading enzymes, the presence of a structural signature associated with the non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of making enoxaparin that includes β-eliminative cleavage with a benzyl ester and depolymerization;

making a determination about the enoxaparin sample based upon a comparison of the determination of the presence of a structural signature associated with the non naturally occurring sugar associated with peak 9 to a reference standard for enoxaparin, and

comparing the structural signature determination to a reference database of structural signatures for enoxaparin,

to thereby analyze the enoxaparin sample.

15. A method for analyzing an enoxaparin sample for the presence or amount of a non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of

making enoxaparin that included β-eliminative cleavage with a benzyl ester and depolymerization, comprising:

providing an enoxaparin sample that has been exhaustively digested with two or more heparin degrading enzymes;

using a separation method to determine, in the enoxaparin sample that has been contacted with two or more heparin degrading enzymes, the presence of a structural signature associated with the non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of making enoxaparin that includes β-eliminative cleavage with a benzyl ester and depolymerization; and

making a determination about the enoxaparin sample based upon a comparison of the determination of the presence of a structural signature associated with the non naturally occurring sugar associated with peak 9 to a reference standard for enoxaparin,

wherein the level of one or more structural signatures associated with the non naturally occurring sugar associated with peak 9 of FIG. **1** is determined,

to thereby analyze the enoxaparin sample.

16. The method of claim **15**, wherein the level of the structural signature is calculated as the area under the curve or as the percent relative amount of each fraction present in the sample.

17. The method of claim **15**, further comprising determining if the level of the structural signature varies less than 2.5% from the reference standard.

18. The method of claim **17**, further comprising determining if the level of the structural signature varies less than 2.0% from the reference standard.

19. The method of claim **18**, further comprising determining if the quantity of the structural signature varies less than 1.0% from the reference standard.

20. A method for analyzing an enoxaparin sample for the presence or amount of a non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of making enoxaparin that included β-eliminative cleavage with a benzyl ester and depolymerization, comprising:

providing an enoxaparin sample that has been exhaustively digested with two or more heparin degrading enzymes;

using a separation method to determine, in the enoxaparin sample that has been contacted with two or more heparin degrading enzymes, the presence of a structural signature associated with the non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of making enoxaparin that includes β-eliminative cleavage with a benzyl ester and depolymerization;

making a determination about the enoxaparin sample based upon a comparison of the determination of the presence of a structural signature associated with the non naturally occurring sugar associated with peak 9 to a reference standard for enoxaparin,

wherein the level of two or more structural signatures associated with non-naturally occurring sugars that result from a method that includes β-eliminative cleavage with a benzyl ester and depolymerization are detected, and wherein the structural signatures of the non naturally occurring sugars are associated with peaks 9 and 10 of FIG. **1**;

determining the level of the structural signatures associated with the non naturally occurring sugars that result from the method used to make enoxaparin and at least one other structural signature,

wherein the other structural signature is one or more of: $\Delta U_{2S}H_{NS,6S}$; $\Delta U_{2S}H_{NS}$; $\Delta UH_{NS,6S}$; $\Delta U_{2S}H_{NAC,6S}$; ΔUH_{NS}; $\Delta U_{2S}H_{NAC}$; $\Delta UH_{NAC,6S}$; $\Delta UH_{NAc,6S}GH_{NS,3S,6S}$; $\Delta UH_{NS,6S}GH_{NS,3S,6S}$; $\Delta UH_{NS,6S}GH_{NS,3S}$ and $\Delta UH_{NAc,6S}GH_{NS,3S}$; and

determining if $\Delta U_{2S}H_{NS,6S}$ is present at 45-80 mole %,

to thereby analyze the enoxaparin sample.

21. The method of claim **20**, wherein the other structural signature is one or more component associated with peak 1 or peak 8 of FIG. **1**.

22. The method of claim **20**, comprising determining if $\Delta U_{2S}H_{NS,6S}$ is present at 50-75 mole %.

23. The method of claim **20**, comprising determining if $\Delta U_{2S}H_{NS,6S}$ is present at 55-70 mole %.

24. The method of claim **20**, comprising determining if $\Delta U_{2S}H_{NS,6S}$ is present at 60-65 mole %.

25. The method of claim **20**, further comprising determining if $\Delta U_{2S}H_{NS}$ is present at 2-15 mole %.

26. The method of claim **20**, further comprising determining if $\Delta U_{2S}H_{NS}$ is present at 5-10 mole %.

27. The method of claim **20**, further comprising determining if $\Delta U_{2S}H_{NS}$ is present at 6-9 mole %.

28. The method of claim **20**, further comprising determining if $\Delta UH_{NS,6S}$ is present at 5-18 mole %.

29. The method of claim **20**, further comprising determining if $\Delta UH_{NS,6S}$ is present at 7-15 mole %.

30. The method of claim **20**, further comprising determining if $\Delta UH_{NS,6S}$ is present at 10-12 mole %.

31. The method of claim **20**, further comprising determining if $\Delta U_{2S}H_{NAC,6S}$ is present at 0.5-7.5 mole %.

32. The method of claim **20**, further comprising determining if $\Delta U_{2S}H_{NAC,6S}$ is present at 1-5 mole %.

33. The method of claim **20**, further comprising determining if $\Delta U_{2S}H_{NAC,6S}$ is present at 1.5-3 mole %.

34. The method of claim **20**, further comprising determining if ΔUH_{NS} is present at 1-7 mole %.

35. The method of claim **20**, further comprising determining if ΔUH_{NS} is present at 2-5 mole %.

36. The method of claim **20**, further comprising determining if ΔUH_{NS} is present at 3-4 mole %.

37. The method of claim **20**, further comprising determining if $\Delta U_{2S}H_{NAC}$ is present at 0.1-5 mole %.

38. The method of claim **20**, further comprising determining if $\Delta U_{2S}H_{NAC}$ is present at 0.5-3 mole %.

39. The method of claim **20**, further comprising determining if $\Delta U_{2S}H_{NAC}$ is present at 1-2.5 mole %.

40. The method of claim **20**, further comprising determining if $\Delta UH_{NAC,6S}$ is present at 0.1-12 mole %.

41. The method of claim **20**, further comprising determining if $\Delta UH_{NAC,6S}$ is present at 0.5-10 mole %.

42. The method of claim **20**, further comprising determining if $\Delta UH_{NAC,6S}$ is present at 1-6 mole %.

43. A method for analyzing an enoxaparin sample for the presence or amount of a non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of making enoxaparin that included β-eliminative cleavage with a benzyl ester and depolymerization, comprising:

providing an enoxaparin sample that has been exhaustively digested with two or more heparin degrading enzymes;

using a separation method to determine, in the enoxaparin sample that has been contacted with two or more heparin degrading enzymes, the presence of a structural signature associated with the non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of making enoxaparin that includes β-eliminative cleavage with a benzyl ester and depolymerization;

making a determination about the enoxaparin sample based upon a comparison of the determination of the presence of a structural signature associated with the

non naturally occurring sugar associated with peak 9 to a reference standard for enoxaparin; and

determining if the non naturally occurring sugar associated with peak 9 of FIG. **1** is present in a preselected range,

to thereby analyze the enoxaparin sample.

44. The method of claim **43**, wherein the preselected range of the non naturally occurring sugar associated with peak 9 of FIG. **1** is 0.1-2.5 mole %.

45. The method of claim **44**, wherein the preselected range is 0.1 to 1 mole %.

46. The method of claim **43**, further comprising determining if a non naturally occurring sugar associated with peak 10 of Fig. is present in a preselected range of 0.1-2.5 mole %.

47. The method of claim **46**, wherein the preselected range is 0.1 to 1 mole %.

48. The method of claim **46**, wherein the preselected range is 0.3 to 0.7 mole %.

49. A method for analyzing an enoxaparin sample for the presence or amount of a non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of making enoxaparin that included β-eliminative cleavage with a benzyl ester and depolymerization, comprising:

providing an enoxaparin sample that has been exhaustively digested with two or more heparin degrading enzymes;

using a separation method to determine, in the enoxaparin sample that has been contacted with two or more heparin degrading enzymes, the presence of a structural signature associated with the non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of making enoxaparin that includes β-eliminative cleavage with a benzyl ester and depolymerization; and

making a determination about the enoxaparin sample based upon a comparison of the determination of the presence of a structural signature associated with the non naturally occurring sugar associated with peak 9 to a reference standard for enoxaparin,

wherein level of two or more structural signatures associated with non-naturally occurring sugars that result from a method that includes β-eliminative cleavage with a benzyl ester and depolymerization are detected,

wherein the structural signatures of the non naturally occurring sugars are associated with peaks 9 and 10 of FIG. **1**,

wherein the method comprises determining the level of the structural signatures associated with the non naturally occurring sugars that result from the method used to make enoxaparin and at least one other structural signature,

and wherein the other structural signature is one or more of: $\Delta U_{2S}H_{NS,6S}$; $\Delta U_{2S}H_{NS}$; $\Delta UH_{NS,6S}$; $\Delta U_{2S}H_{NAC,6S}$; ΔUH_{NS}; $\Delta U_{2S}H_{NAC}$; $\Delta UH_{NAC,6S}$; $\Delta UH_{NAc,6S}GH_{NS,3S,6S}$; $\Delta UH_{NS,6S}GH_{NS,3S,6S}$; $\Delta UH_{NS,6S}GH_{NS,3S}$ and $\Delta UH_{NAc,6S}GH_{NS,3S}$; and

determining if $\Delta UH_{NAc,6S}GH_{NS,3S,6S}$, $\Delta U\,H_{NAc,6S}GH_{NS,3S}$, $\Delta UH_{NS,6S}GH_{NS,3S,6S}$, $\Delta UH_{NS,6S}GH_{NS,3S}$, or mixtures thereof are present at 1-15 mole %,

to thereby analyze the enoxaparin sample.

50. The method of claim **49**, comprising determining if $\Delta UH_{NAc,6S}GH_{NS,3S,6S}$, $\Delta UH_{NAc,6S}GH_{NS,3S}$, $\Delta UH_{NS,6S}GH_{NS,3S,6S}$, $\Delta UH_{NS,6S}GH_{NS,3S}$, or mixtures thereof are present at 2-10 mole %.

51. The method of claim **49**, comprising determining if $\Delta UH_{NAc,6S}GH_{NS,3S,6S}$, $\Delta UH_{NAc,6S}GH_{NS,3S}$, $\Delta UH_{NS,6S}GH_{NS,3S,6S}$, $\Delta UH_{NS,6S}GH_{NS,3S}$, or mixtures thereof are present at 3-8 mole %.

52. The method of claim **49**, comprising determining if $\Delta UH_{NAc,6S}GH_{NS,3S,6S}$, $\Delta UH_{NAc,6S}GH_{NS,3S}$, $\Delta UH_{NS,6S}GH_{NS,3S,6S}$, $\Delta UH_{NS,6S}GH_{NS,3S}$, or mixtures thereof are present at 5-7 mole %.

53. A method for analyzing an enoxaparin sample for the presence or amount of a non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of making enoxaparin that included β-eliminative cleavage with a benzyl ester and depolymerization, comprising:

providing an enoxaparin sample that has been exhaustively digested with two or more heparin degrading enzymes;

using a separation method to determine, in the enoxaparin sample that has been contacted with two or more heparin degrading enzymes, the presence of a structural signature associated with the non naturally occurring sugar associated with peak 9 of FIG. **1** that results from a method of making enoxaparin that includes β-eliminative cleavage with a benzyl ester and depolymerization;

making a determination about the enoxaparin sample based upon a comparison of the determination of the presence of a structural signature associated with the non naturally occurring sugar associated with peak 9 to a reference standard for enoxaparin; and

selecting a batch of enoxaparin based upon a comparison of the determination of the presence of the structural signature associated with the non naturally occurring sugar associated with peak 9 of FIG. **1** to a reference standard for enoxaparin,

to thereby analyze the enoxaparin sample.

54. The method of claims **1**, **3**, **6**, **7**, **8**, **10**, **11**, **14**, **20** or **43**, wherein the structural signature is determined using high performance liquid chromatography (HPLC).

55. The method of claims **1**, **3**, **6**, **7**, **8**, **10**, **11**, **14**, **15**, **20**, **43**, **49** or **53**, wherein the structural signature is determined using NMR.

56. The method of claims **1**, **3**, **6**, **7**, **8**, **10**, **11**, **14**, **15**, **20**, **43**, **49** or **53**, wherein the structural signature is determined using CE.

57. The method of claims **1**, **3**, **6**, **7**, **8**, **10**, **11**, **14**, **15**, **20**, **43**, **49** or **53**, wherein the structural signature is determined using MALDI-MS.

58. The method of claims **1**, **3**, **6**, **7**, **8**, **10**, **11**, **14**, **15**, **20**, **43**, **49** or **53**, wherein the structural signature is determined using ESI-MS.

59. The method of claims **1**, **3**, **6**, **7**, **8**, **10**, **11**, **14**, **15**, **20**, **43**, **49** or **53**, wherein the structural signature is determined using FPLC.

60. The method of claims **1**, **3**, **6**, **7**, **8**, **10**, **11**, **14**, **15**, **20**, **43**, **49** or **53**, wherein the structural signature is determined using one or more of fluorometry, ELISA, chromatogenic assays, and colorimetric assays.

61. The method of claims **1**, **3**, **6**, **7**, **8**, **10**, **11**, **14**, **15**, **20**, **43**, **49** or **53**, wherein the heparin degrading enzymes are selected from the group consisting of heparinase I, heparinase II, heparinase III.

62. The method of claims **1**, **3**, **6**, **7**, **8**, **10**, **11**, **14**, **15**, **20**, **43**, **49** or **53**, wherein the sample is contacted with heparinase I, heparinase II, and heparinase III.

63. The method of claims **1**, **3**, **6**, **7**, **8**, **10**, **11**, **14**, **15**, **20**, **43**, **49** or **53**, further comprising detecting one or more biological activities of the sample.

64. The method of claims **1**, **3**, **6**, **7**, **8**, **10**, **11**, **14**, **15**, **20**, **43**, **49** or **53**, wherein the sample has one or more of the following properties: anti-Xa activity of about 100 IU/mg; a molecular weight distribution of less than or equal to 20% are <2000 Da species, greater than or equal to 68% are 2000-8000 Da

species, and less than or equal to 18% are >8000 Da species; an average molecular weight of about 4500 Da; and a pH of 5.5-7.5.

65. The method of claims **11**, **15**, **20**, or **49**, wherein two or more structural signatures associated with non-naturally occurring sugars that result from a method that includes β-eliminative cleavage with a benzyl ester and depolymerization are detected.

66. The method of claims **1**, **3**, **6**, **7**, **8**, **10**, **11**, **14**, **15**, **20**, **43**, **49** or **53**, wherein the structural signature is a di- or tetrasaccharide.

67. The method of claim **65**, wherein the structural signatures are associated with peaks 9 and 10 of FIG. **1**.

68. The method of claim **67**, wherein the method comprises determining the presence of the structural signatures associated with the non naturally occurring sugars that result from the method used to make enoxaparin and at least one other structural signature.

69. The method of claim **68**, wherein the other structural signature is one or more component associated with peak 1 or peak 8 of FIG. **1**.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,575,886 B2
APPLICATION NO. : 10/386402
DATED : August 18, 2009
INVENTOR(S) : Venkataraman et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

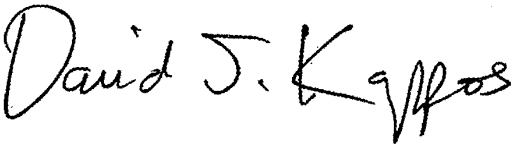
On the Title Page:

The first or sole Notice should read --

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 1447 days.

Signed and Sealed this

Seventh Day of September, 2010



David J. Kappos
Director of the United States Patent and Trademark Office

TITLE 35—PATENTS
PART III—PATENTS AND PROTECTION OF PATENT RIGHTS
CHAPTER 28—INFRINGEMENT OF PATENTS

§ 271. Infringement of patent

(a) Except as otherwise provided in this title, whoever without authority makes, uses, offers to sell, or sells any patented invention, within the United States or imports into the United States any patented invention during the term of the patent therefor, infringes the patent.

(b) Whoever actively induces infringement of a patent shall be liable as an infringer.

(c) Whoever offers to sell or sells within the United States or imports into the United States a component of a patented machine, manufacture, combination or composition, or a material or apparatus for use in practicing a patented process, constituting a material part of the invention, knowing the same to be especially made or especially adapted for use in an infringement of such patent, and not a staple article or commodity of commerce suitable for substantial noninfringing use, shall be liable as a contributory infringer.

(d) No patent owner otherwise entitled to relief for infringement or contributory infringement of a patent shall be denied relief or deemed guilty of misuse or illegal extension of the patent right by reason of his having done one or more of the following: (1) derived revenue from acts which if performed by another without his consent would constitute contributory infringement of the patent; (2) licensed or authorized another to perform acts which if performed without his consent would constitute contributory infringement of the patent; (3) sought to enforce his patent rights against infringement or contributory infringement; (4) refused to license or use any rights to the patent; or (5) conditioned the license of any rights to the patent or the sale of the patented product on the acquisition of a license to rights in another patent or purchase of a separate product, unless, in view of the circumstances, the patent owner has market power in the relevant market for the patent or patented product on which the license or sale is conditioned.

(e)(1) It shall not be an act of infringement to make, use, offer to sell, or sell within the United States or import into the United States a patented invention (other than a new animal drug or veterinary biological product (as those terms are used in the Federal Food, Drug, and Cosmetic Act and the Act of March 4, 1913) which is primarily manufactured using recombinant DNA, recombinant RNA, hybridoma technology, or other processes involving site specific genetic manipulation techniques) solely for uses reasonably related to the development and

submission of information under a Federal law which regulates the manufacture, use, or sale of drugs or veterinary biological products.

(2) It shall be an act of infringement to submit—

(A) an application under section 505(j) of the Federal Food, Drug, and Cosmetic Act or described in section 505(b)(2) of such Act for a drug claimed in a patent or the use of which is claimed in a patent,

(B) an application under section 512 of such Act or under the Act of March 4, 1913 (21 U.S.C. 151–158) for a drug or veterinary biological product which is not primarily manufactured using recombinant DNA, recombinant RNA, hybridoma technology, or other processes involving site specific genetic manipulation techniques and which is claimed in a patent or the use of which is claimed in a patent, or

(C)(i) with respect to a patent that is identified in the list of patents described in section 351(l)(3) of the Public Health Service Act (including as provided under section 351(l)(7) of such Act), an application seeking approval of a biological product, or

(ii) if the applicant for the application fails to provide the application and information required under section 351(l)(2)(A) of such Act, an application seeking approval of a biological product for a patent that could be identified pursuant to section 351(l)(3)(A)(i) of such Act,

if the purpose of such submission is to obtain approval under such Act to engage in the commercial manufacture, use, or sale of a drug, veterinary biological product, or biological product claimed in a patent or the use of which is claimed in a patent before the expiration of such patent.

(3) In any action for patent infringement brought under this section, no injunctive or other relief may be granted which would prohibit the making, using, offering to sell, or selling within the United States or importing into the United States of a patented invention under paragraph (1).

(4) For an act of infringement described in paragraph (2)—

(A) the court shall order the effective date of any approval of the drug or veterinary biological product involved in the infringement to be a date which is not earlier than the date of the expiration of the patent which has been infringed,

(B) injunctive relief may be granted against an infringer to prevent the commercial manufacture, use, offer to sell, or sale within the United States or importation into the United States of an approved drug, veterinary biological product, or biological product,

(C) damages or other monetary relief may be awarded against an infringer only if there has been commercial manufacture, use, offer to sell, or sale within

the United States or importation into the United States of an approved drug, veterinary biological product, or biological product, and

(D) the court shall order a permanent injunction prohibiting any infringement of the patent by the biological product involved in the infringement until a date which is not earlier than the date of the expiration of the patent that has been infringed under paragraph (2)(C), provided the patent is the subject of a final court decision, as defined in section 351(k)(6) of the Public Health Service Act, in an action for infringement of the patent under section 351(l)(6) of such Act, and the biological product has not yet been approved because of section 351(k)(7) of such Act.

The remedies prescribed by subparagraphs (A), (B), (C), and (D) are the only remedies which may be granted by a court for an act of infringement described in paragraph (2), except that a court may award attorney fees under section 285.

(5) Where a person has filed an application described in paragraph (2) that includes a certification under subsection (b)(2)(A)(iv) or (j)(2)(A)(vii)(IV) of section 505 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 355), and neither the owner of the patent that is the subject of the certification nor the holder of the approved application under subsection (b) of such section for the drug that is claimed by the patent or a use of which is claimed by the patent brought an action for infringement of such patent before the expiration of 45 days after the date on which the notice given under subsection (b)(3) or (j)(2)(B) of such section was received, the courts of the United States shall, to the extent consistent with the Constitution, have subject matter jurisdiction in any action brought by such person under section 2201 of title 28 for a declaratory judgment that such patent is invalid or not infringed.

(6)(A) Subparagraph (B) applies, in lieu of paragraph (4), in the case of a patent—

(i) that is identified, as applicable, in the list of patents described in section 351(l)(4) of the Public Health Service Act or the lists of patents described in section 351(l)(5)(B) of such Act with respect to a biological product; and

(ii) for which an action for infringement of the patent with respect to the biological product—

(I) was brought after the expiration of the 30-day period described in subparagraph (A) or (B), as applicable, of section 351(l)(6) of such Act; or

(II) was brought before the expiration of the 30-day period described in subclause (I), but which was dismissed without prejudice or was not prosecuted to judgment in good faith.

(B) In an action for infringement of a patent described in subparagraph (A), the sole and exclusive remedy that may be granted by a court, upon a finding that the making, using, offering to sell, selling, or importation into the United States of the biological product that is the subject of the action infringed the patent, shall be a reasonable royalty.

(C) The owner of a patent that should have been included in the list described in section 351(l)(3)(A) of the Public Health Service Act, including as provided under section 351(l)(7) of such Act for a biological product, but was not timely included in such list, may not bring an action under this section for infringement of the patent with respect to the biological product.

(f)(1) Whoever without authority supplies or causes to be supplied in or from the United States all or a substantial portion of the components of a patented invention, where such components are uncombined in whole or in part, in such manner as to actively induce the combination of such components outside of the United States in a manner that would infringe the patent if such combination occurred within the United States, shall be liable as an infringer.

(2) Whoever without authority supplies or causes to be supplied in or from the United States any component of a patented invention that is especially made or especially adapted for use in the invention and not a staple article or commodity of commerce suitable for substantial noninfringing use, where such component is uncombined in whole or in part, knowing that such component is so made or adapted and intending that such component will be combined outside of the United States in a manner that would infringe the patent if such combination occurred within the United States, shall be liable as an infringer.

(g) Whoever without authority imports into the United States or offers to sell, sells, or uses within the United States a product which is made by a process patented in the United States shall be liable as an infringer, if the importation, offer to sell, sale, or use of the product occurs during the term of such process patent. In an action for infringement of a process patent, no remedy may be granted for infringement on account of the noncommercial use or retail sale of a product unless there is no adequate remedy under this title for infringement on account of the importation or other use, offer to sell, or sale of that product. A product which is made by a patented process will, for purposes of this title, not be considered to be so made after—

(1) it is materially changed by subsequent processes; or

(2) it becomes a trivial and nonessential component of another product.

(h) As used in this section, the term “whoever” includes any State, any instrumentality of a State, and any officer or employee of a State or instrumentality

of a State acting in his official capacity. Any State, and any such instrumentality, officer, or employee, shall be subject to the provisions of this title in the same manner and to the same extent as any nongovernmental entity.

(i) As used in this section, an "offer for sale" or an "offer to sell" by a person other than the patentee, or any designee of the patentee, is that in which the sale will occur before the expiration of the term of the patent.

UNITED STATES CODE

TITLE 21—FOOD AND DRUGS
CHAPTER 9—FEDERAL FOOD, DRUG, AND COSMETIC ACT
SUBCHAPTER III—PROHIBITED ACTS AND PENALTIES

§ 331. Prohibited acts

The following acts and the causing thereof are prohibited:

* * *

(e) The refusal to permit access to or copying of any record as required by section 350a, 350c, 350f (j), 350e, 354, 360bbb–3, 373, 374 (a), 379aa, or 379aa–1 of this title; or the failure to establish or maintain any record, or make any report, required under section 350a, 350c (b), 350f, 350e, 354, 355 (i) or (k), 360b (a)(4)(C), 360b (j), (l) or (m), 360ccc–1 (i), 360e (f), 360i, 360bbb–3, 379aa, 379aa–1, 387i, or 387t of this title or the refusal to permit access to or verification or copying of any such required record; or the violation of any recordkeeping requirement under section 2223 1 of this title (except when such violation is committed by a farm).

* * *

SUBCHAPTER V—DRUGS AND DEVICES
Part A—Drugs and Devices

§ 355. New drugs

(a) Necessity of effective approval of application

No person shall introduce or deliver for introduction into interstate commerce any new drug, unless an approval of an application filed pursuant to subsection (b) or (j) of this section is effective with respect to such drug.

(b) Filing application; contents

(1) Any person may file with the Secretary an application with respect to any drug subject to the provisions of subsection (a) of this section. Such person shall submit to the Secretary as a part of the application (A) full reports of investigations which have been made to show whether or not such drug is safe for use and whether such drug is effective in use; (B) a full list of the articles used as components of such drug; (C) a full statement of the composition of such drug; (D)

a full description of the methods used in, and the facilities and controls used for, the manufacture, processing, and packing of such drug; (E) such samples of such drug and of the articles used as components thereof as the Secretary may require; (F) specimens of the labeling proposed to be used for such drug, and (G) any assessments required under section 355c of this title. The applicant shall file with the application the patent number and the expiration date of any patent which claims the drug for which the applicant submitted the application or which claims a method of using such drug and with respect to which a claim of patent infringement could reasonably be asserted if a person not licensed by the owner engaged in the manufacture, use, or sale of the drug. If an application is filed under this subsection for a drug and a patent which claims such drug or a method of using such drug is issued after the filing date but before approval of the application, the applicant shall amend the application to include the information required by the preceding sentence. Upon approval of the application, the Secretary shall publish information submitted under the two preceding sentences. The Secretary shall, in consultation with the Director of the National Institutes of Health and with representatives of the drug manufacturing industry, review and develop guidance, as appropriate, on the inclusion of women and minorities in clinical trials required by clause (A).

(2) An application submitted under paragraph (1) for a drug for which the investigations described in clause (A) of such paragraph and relied upon by the applicant for approval of the application were not conducted by or for the applicant and for which the applicant has not obtained a right of reference or use from the person by or for whom the investigations were conducted shall also include—

(A) a certification, in the opinion of the applicant and to the best of his knowledge, with respect to each patent which claims the drug for which such investigations were conducted or which claims a use for such drug for which the applicant is seeking approval under this subsection and for which information is required to be filed under paragraph (1) or subsection (c) of this section—

(i) that such patent information has not been filed,

(ii) that such patent has expired,

(iii) of the date on which such patent will expire, or

(iv) that such patent is invalid or will not be infringed by the manufacture, use, or sale of the new drug for which the application is submitted; and

(B) if with respect to the drug for which investigations described in paragraph (1)(A) were conducted information was filed under paragraph (1) or subsection (c)

of this section for a method of use patent which does not claim a use for which the applicant is seeking approval under this subsection, a statement that the method of use patent does not claim such a use.

(3) NOTICE OF OPINION THAT PATENT IS INVALID OR WILL NOT BE INFRINGED.—

(A) AGREEMENT TO GIVE NOTICE.— An applicant that makes a certification described in paragraph (2)(A)(iv) shall include in the application a statement that the applicant will give notice as required by this paragraph.

(B) TIMING OF NOTICE.— An applicant that makes a certification described in paragraph (2)(A)(iv) shall give notice as required under this paragraph—

(i) if the certification is in the application, not later than 20 days after the date of the postmark on the notice with which the Secretary informs the applicant that the application has been filed; or

(ii) if the certification is in an amendment or supplement to the application, at the time at which the applicant submits the amendment or supplement, regardless of whether the applicant has already given notice with respect to another such certification contained in the application or in an amendment or supplement to the application.

(C) RECIPIENTS OF NOTICE.— An applicant required under this paragraph to give notice shall give notice to—

(i) each owner of the patent that is the subject of the certification (or a representative of the owner designated to receive such a notice); and

(ii) the holder of the approved application under this subsection for the drug that is claimed by the patent or a use of which is claimed by the patent (or a representative of the holder designated to receive such a notice).

(D) CONTENTS OF NOTICE.— A notice required under this paragraph shall—

(i) state that an application that contains data from bioavailability or bioequivalence studies has been submitted under this subsection for the drug with respect to which the certification is made to obtain approval to engage in the commercial manufacture, use, or sale of the drug before the expiration of the patent referred to in the certification; and

(ii) include a detailed statement of the factual and legal basis of the opinion of the applicant that the patent is invalid or will not be infringed.

(4)(A) An applicant may not amend or supplement an application referred to in paragraph (2) to seek approval of a drug that is a different drug than the drug identified in the application as submitted to the Secretary.

(B) With respect to the drug for which such an application is submitted, nothing in this subsection or subsection (c)(3) of this section prohibits an applicant from amending or supplementing the application to seek approval of a different strength.

(5)(A) The Secretary shall issue guidance for the individuals who review applications submitted under paragraph (1) or under section 262 of title 42, which shall relate to promptness in conducting the review, technical excellence, lack of bias and conflict of interest, and knowledge of regulatory and scientific standards, and which shall apply equally to all individuals who review such applications.

(B) The Secretary shall meet with a sponsor of an investigation or an applicant for approval for a drug under this subsection or section 262 of title 42 if the sponsor or applicant makes a reasonable written request for a meeting for the purpose of reaching agreement on the design and size of clinical trials intended to form the primary basis of an effectiveness claim or, with respect to an applicant for approval of a biological product under section 262 (k) of title 42, any necessary clinical study or studies. The sponsor or applicant shall provide information necessary for discussion and agreement on the design and size of the clinical trials. Minutes of any such meeting shall be prepared by the Secretary and made available to the sponsor or applicant upon request.

(C) Any agreement regarding the parameters of the design and size of clinical trials of a new drug under this paragraph that is reached between the Secretary and a sponsor or applicant shall be reduced to writing and made part of the administrative record by the Secretary. Such agreement shall not be changed after the testing begins, except—

(i) with the written agreement of the sponsor or applicant; or

(ii) pursuant to a decision, made in accordance with subparagraph (D) by the director of the reviewing division, that a substantial scientific issue essential to determining the safety or effectiveness of the drug has been identified after the testing has begun.

(D) A decision under subparagraph (C)(ii) by the director shall be in writing and the Secretary shall provide to the sponsor or applicant an opportunity for a meeting at which the director and the sponsor or applicant will be present and at which the director will document the scientific issue involved.

(E) The written decisions of the reviewing division shall be binding upon, and may not directly or indirectly be changed by, the field or compliance division personnel unless such field or compliance division personnel demonstrate to the reviewing division why such decision should be modified.

(F) No action by the reviewing division may be delayed because of the unavailability of information from or action by field personnel unless the

reviewing division determines that a delay is necessary to assure the marketing of a safe and effective drug.

(G) For purposes of this paragraph, the reviewing division is the division responsible for the review of an application for approval of a drug under this subsection or section 262 of title 42 (including all scientific and medical matters, chemistry, manufacturing, and controls).

(6) An application submitted under this subsection shall be accompanied by the certification required under section 282 (j)(5)(B) of title 42. Such certification shall not be considered an element of such application.

(c) Period for approval of application; period for, notice, and expedition of hearing; period for issuance of order

(1) Within one hundred and eighty days after the filing of an application under subsection (b) of this section, or such additional period as may be agreed upon by the Secretary and the applicant, the Secretary shall either—

(A) approve the application if he then finds that none of the grounds for denying approval specified in subsection (d) of this section applies, or

(B) give the applicant notice of an opportunity for a hearing before the Secretary under subsection (d) of this section on the question whether such application is approvable. If the applicant elects to accept the opportunity for hearing by written request within thirty days after such notice, such hearing shall commence not more than ninety days after the expiration of such thirty days unless the Secretary and the applicant otherwise agree. Any such hearing shall thereafter be conducted on an expedited basis and the Secretary's order thereon shall be issued within ninety days after the date fixed by the Secretary for filing final briefs.

(2) If the patent information described in subsection (b) of this section could not be filed with the submission of an application under subsection (b) of this section because the application was filed before the patent information was required under subsection (b) of this section or a patent was issued after the application was approved under such subsection, the holder of an approved application shall file with the Secretary the patent number and the expiration date of any patent which claims the drug for which the application was submitted or which claims a method of using such drug and with respect to which a claim of patent infringement could reasonably be asserted if a person not licensed by the owner engaged in the manufacture, use, or sale of the drug. If the holder of an approved application could not file patent information under subsection (b) of this section because it was not required at the time the application was approved, the holder shall file such information under this subsection not later than thirty days after September 24,

1984, and if the holder of an approved application could not file patent information under subsection (b) of this section because no patent had been issued when an application was filed or approved, the holder shall file such information under this subsection not later than thirty days after the date the patent involved is issued. Upon the submission of patent information under this subsection, the Secretary shall publish it.

* * *

(d) Grounds for refusing application; approval of application; "substantial evidence" defined

If the Secretary finds, after due notice to the applicant in accordance with subsection (c) of this section and giving him an opportunity for a hearing, in accordance with said subsection, that (1) the investigations, reports of which are required to be submitted to the Secretary pursuant to subsection (b) of this section, do not include adequate tests by all methods reasonably applicable to show whether or not such drug is safe for use under the conditions prescribed, recommended, or suggested in the proposed labeling thereof; (2) the results of such tests show that such drug is unsafe for use under such conditions or do not show that such drug is safe for use under such conditions; (3) the methods used in, and the facilities and controls used for, the manufacture, processing, and packing of such drug are inadequate to preserve its identity, strength, quality, and purity; (4) upon the basis of the information submitted to him as part of the application, or upon the basis of any other information before him with respect to such drug, he has insufficient information to determine whether such drug is safe for use under such conditions; or (5) evaluated on the basis of the information submitted to him as part of the application and any other information before him with respect to such drug, there is a lack of substantial evidence that the drug will have the effect it purports or is represented to have under the conditions of use prescribed, recommended, or suggested in the proposed labeling thereof; or (6) the application failed to contain the patent information prescribed by subsection (b) of this section; or (7) based on a fair evaluation of all material facts, such labeling is false or misleading in any particular; he shall issue an order refusing to approve the application. If, after such notice and opportunity for hearing, the Secretary finds that clauses (1) through (6) do not apply, he shall issue an order approving the application. As used in this subsection and subsection (e) of this section, the term "substantial evidence" means evidence consisting of adequate and well-controlled investigations, including clinical investigations, by experts qualified by scientific training and experience to evaluate the effectiveness of the drug involved, on the basis of which it could fairly and responsibly be concluded by such experts that the

drug will have the effect it purports or is represented to have under the conditions of use prescribed, recommended, or suggested in the labeling or proposed labeling thereof. If the Secretary determines, based on relevant science, that data from one adequate and well-controlled clinical investigation and confirmatory evidence (obtained prior to or after such investigation) are sufficient to establish effectiveness, the Secretary may consider such data and evidence to constitute substantial evidence for purposes of the preceding sentence.

(e) Withdrawal of approval; grounds; immediate suspension upon finding imminent hazard to public health

The Secretary shall, after due notice and opportunity for hearing to the applicant, withdraw approval of an application with respect to any drug under this section if the Secretary finds (1) that clinical or other experience, tests, or other scientific data show that such drug is unsafe for use under the conditions of use upon the basis of which the application was approved; (2) that new evidence of clinical experience, not contained in such application or not available to the Secretary until after such application was approved, or tests by new methods, or tests by methods not deemed reasonably applicable when such application was approved, evaluated together with the evidence available to the Secretary when the application was approved, shows that such drug is not shown to be safe for use under the conditions of use upon the basis of which the application was approved; or (3) on the basis of new information before him with respect to such drug, evaluated together with the evidence available to him when the application was approved, that there is a lack of substantial evidence that the drug will have the effect it purports or is represented to have under the conditions of use prescribed, recommended, or suggested in the labeling thereof; or (4) the patent information prescribed by subsection (c) of this section was not filed within thirty days after the receipt of written notice from the Secretary specifying the failure to file such information; or (5) that the application contains any untrue statement of a material fact: Provided, That if the Secretary (or in his absence the officer acting as Secretary) finds that there is an imminent hazard to the public health, he may suspend the approval of such application immediately, and give the applicant prompt notice of his action and afford the applicant the opportunity for an expedited hearing under this subsection; but the authority conferred by this proviso to suspend the approval of an application shall not be delegated. The Secretary may also, after due notice and opportunity for hearing to the applicant, withdraw the approval of an application submitted under subsection (b) or (j) of this section with respect to any drug under this section if the Secretary finds (1) that the applicant has failed to establish a system for maintaining required records, or has repeatedly or deliberately failed to maintain such records or to make required

reports, in accordance with a regulation or order under subsection (k) of this section or to comply with the notice requirements of section 360 (k)(2) of this title, or the applicant has refused to permit access to, or copying or verification of, such records as required by paragraph (2) of such subsection; or (2) that on the basis of new information before him, evaluated together with the evidence before him when the application was approved, the methods used in, or the facilities and controls used for, the manufacture, processing, and packing of such drug are inadequate to assure and preserve its identity, strength, quality, and purity and were not made adequate within a reasonable time after receipt of written notice from the Secretary specifying the matter complained of; or (3) that on the basis of new information before him, evaluated together with the evidence before him when the application was approved, the labeling of such drug, based on a fair evaluation of all material facts, is false or misleading in any particular and was not corrected within a reasonable time after receipt of written notice from the Secretary specifying the matter complained of. Any order under this subsection shall state the findings upon which it is based. The Secretary may withdraw the approval of an application submitted under this section, or suspend the approval of such an application, as provided under this subsection, without first ordering the applicant to submit an assessment of the approved risk evaluation and mitigation strategy for the drug under section 355–1 (g)(2)(D) of this title.

* * *

(i) Exemptions of drugs for research; discretionary and mandatory conditions; direct reports to Secretary

(1) The Secretary shall promulgate regulations for exempting from the operation of the foregoing subsections of this section drugs intended solely for investigational use by experts qualified by scientific training and experience to investigate the safety and effectiveness of drugs. Such regulations may, within the discretion of the Secretary, among other conditions relating to the protection of the public health, provide for conditioning such exemption upon—

(A) the submission to the Secretary, before any clinical testing of a new drug is undertaken, of reports, by the manufacturer or the sponsor of the investigation of such drug, of preclinical tests (including tests on animals) of such drug adequate to justify the proposed clinical testing;

(B) the manufacturer or the sponsor of the investigation of a new drug proposed to be distributed to investigators for clinical testing obtaining a signed agreement from each of such investigators that patients to whom the drug is administered will be under his personal supervision, or under the supervision of

investigators responsible to him, and that he will not supply such drug to any other investigator, or to clinics, for administration to human beings;

(C) the establishment and maintenance of such records, and the making of such reports to the Secretary, by the manufacturer or the sponsor of the investigation of such drug, of data (including but not limited to analytical reports by investigators) obtained as the result of such investigational use of such drug, as the Secretary finds will enable him to evaluate the safety and effectiveness of such drug in the event of the filing of an application pursuant to subsection (b) of this section; and

(D) the submission to the Secretary by the manufacturer or the sponsor of the investigation of a new drug of a statement of intent regarding whether the manufacturer or sponsor has plans for assessing pediatric safety and efficacy.

* * *

(j) Abbreviated new drug applications

(1) Any person may file with the Secretary an abbreviated application for the approval of a new drug.

(2)(A) An abbreviated application for a new drug shall contain—

(i) information to show that the conditions of use prescribed, recommended, or suggested in the labeling proposed for the new drug have been previously approved for a drug listed under paragraph (7) (hereinafter in this subsection referred to as a "listed drug");

(ii)(I) if the listed drug referred to in clause (i) has only one active ingredient, information to show that the active ingredient of the new drug is the same as that of the listed drug;

(II) if the listed drug referred to in clause (i) has more than one active ingredient, information to show that the active ingredients of the new drug are the same as those of the listed drug, or

(III) if the listed drug referred to in clause (i) has more than one active ingredient and if one of the active ingredients of the new drug is different and the application is filed pursuant to the approval of a petition filed under subparagraph (C), information to show that the other active ingredients of the new drug are the same as the active ingredients of the listed drug, information to show that the different active ingredient is an active ingredient of a listed drug or of a drug which does not meet the requirements of section 321 (p) of this title, and such other information respecting the different active ingredient with respect to which the petition was filed as the Secretary may require;

(iii) information to show that the route of administration, the dosage form, and the strength of the new drug are the same as those of the listed drug referred to in clause (i) or, if the route of administration, the dosage form, or the strength of the new drug is different and the application is filed pursuant to the approval of a petition filed under subparagraph (C), such information respecting the route of administration, dosage form, or strength with respect to which the petition was filed as the Secretary may require;

(iv) information to show that the new drug is bioequivalent to the listed drug referred to in clause (i), except that if the application is filed pursuant to the approval of a petition filed under subparagraph (C), information to show that the active ingredients of the new drug are of the same pharmacological or therapeutic class as those of the listed drug referred to in clause (i) and the new drug can be expected to have the same therapeutic effect as the listed drug when administered to patients for a condition of use referred to in clause (i);

(v) information to show that the labeling proposed for the new drug is the same as the labeling approved for the listed drug referred to in clause (i) except for changes required because of differences approved under a petition filed under subparagraph (C) or because the new drug and the listed drug are produced or distributed by different manufacturers;

(vi) the items specified in clauses (B) through (F) of subsection (b)(1) of this section;

(vii) a certification, in the opinion of the applicant and to the best of his knowledge, with respect to each patent which claims the listed drug referred to in clause (i) or which claims a use for such listed drug for which the applicant is seeking approval under this subsection and for which information is required to be filed under subsection (b) or (c) of this section—

(I) that such patent information has not been filed,

(II) that such patent has expired,

(III) of the date on which such patent will expire, or

(IV) that such patent is invalid or will not be infringed by the manufacture, use, or sale of the new drug for which the application is submitted; and

(viii) if with respect to the listed drug referred to in clause (i) information was filed under subsection (b) or (c) of this section for a method of use patent which does not claim a use for which the applicant is seeking approval under this subsection, a statement that the method of use patent does not claim such a use.

The Secretary may not require that an abbreviated application contain information in addition to that required by clauses (i) through (viii).

(B) NOTICE OF OPINION THAT PATENT IS INVALID OR WILL NOT BE INFRINGED.—

(i) AGREEMENT TO GIVE NOTICE.— An applicant that makes a certification described in subparagraph (A)(vii)(IV) shall include in the application a statement that the applicant will give notice as required by this subparagraph.

(ii) TIMING OF NOTICE.— An applicant that makes a certification described in subparagraph (A)(vii)(IV) shall give notice as required under this subparagraph—

(I) if the certification is in the application, not later than 20 days after the date of the postmark on the notice with which the Secretary informs the applicant that the application has been filed; or

(II) if the certification is in an amendment or supplement to the application, at the time at which the applicant submits the amendment or supplement, regardless of whether the applicant has already given notice with respect to another such certification contained in the application or in an amendment or supplement to the application.

(iii) RECIPIENTS OF NOTICE.— An applicant required under this subparagraph to give notice shall give notice to—

(I) each owner of the patent that is the subject of the certification (or a representative of the owner designated to receive such a notice); and

(II) the holder of the approved application under subsection (b) of this section for the drug that is claimed by the patent or a use of which is claimed by the patent (or a representative of the holder designated to receive such a notice).

(iv) CONTENTS OF NOTICE.— A notice required under this subparagraph shall—

(I) state that an application that contains data from bioavailability or bioequivalence studies has been submitted under this subsection for the drug with respect to which the certification is made to obtain approval to engage in the commercial manufacture, use, or sale of the drug before the expiration of the patent referred to in the certification; and

(II) include a detailed statement of the factual and legal basis of the opinion of the applicant that the patent is invalid or will not be infringed.

(C) If a person wants to submit an abbreviated application for a new drug which has a different active ingredient or whose route of administration, dosage form, or strength differ from that of a listed drug, such person shall submit a petition to the Secretary seeking permission to file such an application. The Secretary shall

approve or disapprove a petition submitted under this subparagraph within ninety days of the date the petition is submitted. The Secretary shall approve such a petition unless the Secretary finds—

(i) that investigations must be conducted to show the safety and effectiveness of the drug or of any of its active ingredients, the route of administration, the dosage form, or strength which differ from the listed drug; or

(ii) that any drug with a different active ingredient may not be adequately evaluated for approval as safe and effective on the basis of the information required to be submitted in an abbreviated application.

(D)(i) An applicant may not amend or supplement an application to seek approval of a drug referring to a different listed drug from the listed drug identified in the application as submitted to the Secretary.

(ii) With respect to the drug for which an application is submitted, nothing in this subsection prohibits an applicant from amending or supplementing the application to seek approval of a different strength.

(iii) Within 60 days after December 8, 2003, the Secretary shall issue guidance defining the term “listed drug” for purposes of this subparagraph.

* * *

(4) Subject to paragraph (5), the Secretary shall approve an application for a drug unless the Secretary finds—

(A) the methods used in, or the facilities and controls used for, the manufacture, processing, and packing of the drug are inadequate to assure and preserve its identity, strength, quality, and purity;

(B) information submitted with the application is insufficient to show that each of the proposed conditions of use have been previously approved for the listed drug referred to in the application;

(C)(i) if the listed drug has only one active ingredient, information submitted with the application is insufficient to show that the active ingredient is the same as that of the listed drug;

(ii) if the listed drug has more than one active ingredient, information submitted with the application is insufficient to show that the active ingredients are the same as the active ingredients of the listed drug, or

(iii) if the listed drug has more than one active ingredient and if the application is for a drug which has an active ingredient different from the listed drug, information submitted with the application is insufficient to show—

(I) that the other active ingredients are the same as the active ingredients of the listed drug, or

(II) that the different active ingredient is an active ingredient of a listed drug or a drug which does not meet the requirements of section 321 (p) of this title,

or no petition to file an application for the drug with the different ingredient was approved under paragraph (2)(C);

(D)(i) if the application is for a drug whose route of administration, dosage form, or strength of the drug is the same as the route of administration, dosage form, or strength of the listed drug referred to in the application, information submitted in the application is insufficient to show that the route of administration, dosage form, or strength is the same as that of the listed drug, or

(ii) if the application is for a drug whose route of administration, dosage form, or strength of the drug is different from that of the listed drug referred to in the application, no petition to file an application for the drug with the different route of administration, dosage form, or strength was approved under paragraph (2)(C);

(E) if the application was filed pursuant to the approval of a petition under paragraph (2)(C), the application did not contain the information required by the Secretary respecting the active ingredient, route of administration, dosage form, or strength which is not the same;

(F) information submitted in the application is insufficient to show that the drug is bioequivalent to the listed drug referred to in the application or, if the application was filed pursuant to a petition approved under paragraph (2)(C), information submitted in the application is insufficient to show that the active ingredients of the new drug are of the same pharmacological or therapeutic class as those of the listed drug referred to in paragraph (2)(A)(i) and that the new drug can be expected to have the same therapeutic effect as the listed drug when administered to patients for a condition of use referred to in such paragraph;

(G) information submitted in the application is insufficient to show that the labeling proposed for the drug is the same as the labeling approved for the listed drug referred to in the application except for changes required because of differences approved under a petition filed under paragraph (2)(C) or because the drug and the listed drug are produced or distributed by different manufacturers;

(H) information submitted in the application or any other information available to the Secretary shows that (i) the inactive ingredients of the drug are

unsafe for use under the conditions prescribed, recommended, or suggested in the labeling proposed for the drug, or (ii) the composition of the drug is unsafe under such conditions because of the type or quantity of inactive ingredients included or the manner in which the inactive ingredients are included;

(I) the approval under subsection (c) of this section of the listed drug referred to in the application under this subsection has been withdrawn or suspended for grounds described in the first sentence of subsection (e) of this section, the Secretary has published a notice of opportunity for hearing to withdraw approval of the listed drug under subsection (c) of this section for grounds described in the first sentence of subsection (e) of this section, the approval under this subsection of the listed drug referred to in the application under this subsection has been withdrawn or suspended under paragraph (6), or the Secretary has determined that the listed drug has been withdrawn from sale for safety or effectiveness reasons;

(J) the application does not meet any other requirement of paragraph (2)(A); or

(K) the application contains an untrue statement of material fact.

* * *

(k) Records and reports; required information; regulations and orders; access to records

(1) In the case of any drug for which an approval of an application filed under subsection (b) or (j) of this section is in effect, the applicant shall establish and maintain such records, and make such reports to the Secretary, of data relating to clinical experience and other data or information, received or otherwise obtained by such applicant with respect to such drug, as the Secretary may by general regulation, or by order with respect to such application, prescribe on the basis of a finding that such records and reports are necessary in order to enable the Secretary to determine, or facilitate a determination, whether there is or may be ground for invoking subsection (e) of this section. Regulations and orders issued under this subsection and under subsection (i) of this section shall have due regard for the professional ethics of the medical profession and the interests of patients and shall provide, where the Secretary deems it to be appropriate, for the examination, upon request, by the persons to whom such regulations or orders are applicable, of similar information received or otherwise obtained by the Secretary.

(2) Every person required under this section to maintain records, and every person in charge or custody thereof, shall, upon request of an officer or employee

designated by the Secretary, permit such officer or employee at all reasonable times to have access to and copy and verify such records.

* * *

(*o*) Postmarket studies and clinical trials; labeling

(1) In general

A responsible person may not introduce or deliver for introduction into interstate commerce the new drug involved if the person is in violation of a requirement established under paragraph (3) or (4) with respect to the drug.

(2) Definitions

For purposes of this subsection:

(A) Responsible person

The term "responsible person" means a person who—

(i) has submitted to the Secretary a covered application that is pending; or

(ii) is the holder of an approved covered application.

(B) Covered application

The term "covered application" means—

(i) an application under subsection (b) for a drug that is subject to section 353 (b) of this title; and

(ii) an application under section 262 of title 42.

(C) New safety information; serious risk

The terms "new safety information", "serious risk", and "signal of a serious risk" have the meanings given such terms in section 355–1 (b) of this title.

(3) Studies and clinical trials

(A) In general

For any or all of the purposes specified in subparagraph (B), the Secretary may, subject to subparagraph (D), require a responsible person for a drug to conduct a postapproval study or studies of the drug, or a postapproval clinical trial or trials of the drug, on the basis of scientific data deemed appropriate by the Secretary, including information regarding chemically-related or pharmacologically-related drugs.

(B) Purposes of study or clinical trial

The purposes referred to in this subparagraph with respect to a postapproval study or postapproval clinical trial are the following:

(i) To assess a known serious risk related to the use of the drug involved.

(ii) To assess signals of serious risk related to the use of the drug.

(iii) To identify an unexpected serious risk when available data indicates the potential for a serious risk.

(C) Establishment of requirement after approval of covered application

The Secretary may require a postapproval study or studies or postapproval clinical trial or trials for a drug for which an approved covered application is in effect as of the date on which the Secretary seeks to establish such requirement only if the Secretary becomes aware of new safety information.

(D) Determination by Secretary

(i) Postapproval studies

The Secretary may not require the responsible person to conduct a study under this paragraph, unless the Secretary makes a determination that the reports under subsection (k)(1) and the active postmarket risk identification and analysis system as available under subsection (k)(3) will not be sufficient to meet the purposes set forth in subparagraph (B).

(ii) Postapproval clinical trials

The Secretary may not require the responsible person to conduct a clinical trial under this paragraph, unless the Secretary makes a determination that a postapproval study or studies will not be sufficient to meet the purposes set forth in subparagraph (B).

(E) Notification; timetables; periodic reports

(i) Notification

The Secretary shall notify the responsible person regarding a requirement under this paragraph to conduct a postapproval study or clinical trial by the target dates for communication of feedback from the review team to the responsible person regarding proposed labeling and postmarketing study commitments as set forth in the letters described in section 101(c) of the Food and Drug Administration Amendments Act of 2007.

(ii) Timetable; periodic reports

For each study or clinical trial required to be conducted under this paragraph, the Secretary shall require that the responsible person submit

a timetable for completion of the study or clinical trial. With respect to each study required to be conducted under this paragraph or otherwise undertaken by the responsible person to investigate a safety issue, the Secretary shall require the responsible person to periodically report to the Secretary on the status of such study including whether any difficulties in completing the study have been encountered. With respect to each clinical trial required to be conducted under this paragraph or otherwise undertaken by the responsible person to investigate a safety issue, the Secretary shall require the responsible person to periodically report to the Secretary on the status of such clinical trial including whether enrollment has begun, the number of participants enrolled, the expected completion date, whether any difficulties completing the clinical trial have been encountered, and registration information with respect to the requirements under section 282 (j) of title 42. If the responsible person fails to comply with such timetable or violates any other requirement of this subparagraph, the responsible person shall be considered in violation of this subsection, unless the responsible person demonstrates good cause for such noncompliance or such other violation. The Secretary shall determine what constitutes good cause under the preceding sentence.

(F) Dispute resolution

The responsible person may appeal a requirement to conduct a study or clinical trial under this paragraph using dispute resolution procedures established by the Secretary in regulation and guidance.

(4) Safety labeling changes requested by Secretary

(A) New safety information

If the Secretary becomes aware of new safety information that the Secretary believes should be included in the labeling of the drug, the Secretary shall promptly notify the responsible person or, if the same drug approved under subsection (b) is not currently marketed, the holder of an approved application under subsection (j).

(B) Response to notification

Following notification pursuant to subparagraph (A), the responsible person or the holder of the approved application under subsection (j) shall within 30 days—

(i) submit a supplement proposing changes to the approved labeling to reflect the new safety information, including changes to boxed warnings, contraindications, warnings, precautions, or adverse reactions; or

(ii) notify the Secretary that the responsible person or the holder of the approved application under subsection (j) does not believe a labeling change is warranted and submit a statement detailing the reasons why such a change is not warranted.

(C) Review

Upon receipt of such supplement, the Secretary shall promptly review and act upon such supplement. If the Secretary disagrees with the proposed changes in the supplement or with the statement setting forth the reasons why no labeling change is necessary, the Secretary shall initiate discussions to reach agreement on whether the labeling for the drug should be modified to reflect the new safety information, and if so, the contents of such labeling changes.

(D) Discussions

Such discussions shall not extend for more than 30 days after the response to the notification under subparagraph (B), unless the Secretary determines an extension of such discussion period is warranted.

(E) Order

Within 15 days of the conclusion of the discussions under subparagraph (D), the Secretary may issue an order directing the responsible person or the holder of the approved application under subsection (j) to make such a labeling change as the Secretary deems appropriate to address the new safety information. Within 15 days of such an order, the responsible person or the holder of the approved application under subsection (j) shall submit a supplement containing the labeling change.

(F) Dispute resolution

Within 5 days of receiving an order under subparagraph (E), the responsible person or the holder of the approved application under subsection (j) may appeal using dispute resolution procedures established by the Secretary in regulation and guidance.

(G) Violation

If the responsible person or the holder of the approved application under subsection (j) has not submitted a supplement within 15 days of the date of such order under subparagraph (E), and there is no appeal or dispute resolution proceeding pending, the responsible person or holder shall be considered to be in violation of this subsection. If at the conclusion of any dispute resolution procedures the Secretary determines that a supplement must be submitted and such a supplement is not submitted within 15 days of

the date of that determination, the responsible person or holder shall be in violation of this subsection.

(H) Public health threat

Notwithstanding subparagraphs (A) through (F), if the Secretary concludes that such a labeling change is necessary to protect the public health, the Secretary may accelerate the timelines in such subparagraphs.

(I) Rule of construction

This paragraph shall not be construed to affect the responsibility of the responsible person or the holder of the approved application under subsection (j) to maintain its label in accordance with existing requirements, including subpart B of part 201 and sections 314.70 and 601.12 of title 21, Code of Federal Regulations (or any successor regulations).

(5) Non-delegation

Determinations by the Secretary under this subsection for a drug shall be made by individuals at or above the level of individuals empowered to approve a drug (such as division directors within the Center for Drug Evaluation and Research).

* * *

§ 356. Expedited approval of drugs for serious or life-threatening diseases or conditions

(a) Designation of a drug as a breakthrough therapy

(1) In general

The Secretary shall, at the request of the sponsor of a drug, expedite the development and review of such drug if the drug is intended, alone or in combination with 1 or more other drugs, to treat a serious or life-threatening disease or condition and preliminary clinical evidence indicates that the drug may demonstrate substantial improvement over existing therapies on 1 or more clinically significant endpoints, such as substantial treatment effects observed early in clinical development. (In this section, such a drug is referred to as a "breakthrough therapy".)

(2) Request for designation

The sponsor of a drug may request the Secretary to designate the drug as a breakthrough therapy. A request for the designation may be made concurrently with, or at any time after, the submission of an application for the investigation of the drug under section 355(i) of this title or section 262(a)(3) of title 42.

(3) Designation

(A) In general

Not later than 60 calendar days after the receipt of a request under paragraph (2), the Secretary shall determine whether the drug that is the subject of the request meets the criteria described in paragraph (1). If the Secretary finds that the drug meets the criteria, the Secretary shall designate the drug as a breakthrough therapy and shall take such actions as are appropriate to expedite the development and review of the application for approval of such drug.

(B) Actions

The actions to expedite the development and review of an application under subparagraph (A) may include, as appropriate—

(i) holding meetings with the sponsor and the review team throughout the development of the drug;

(ii) providing timely advice to, and interactive communication with, the sponsor regarding the development of the drug to ensure that the development program to gather the nonclinical and clinical data necessary for approval is as efficient as practicable;

(iii) involving senior managers and experienced review staff, as appropriate, in a collaborative, cross-disciplinary review;

(iv) assigning a cross-disciplinary project lead for the Food and Drug Administration review team to facilitate an efficient review of the development program and to serve as a scientific liaison between the review team and the sponsor; and

(v) taking steps to ensure that the design of the clinical trials is as efficient as practicable, when scientifically appropriate, such as by minimizing the number of patients exposed to a potentially less efficacious treatment.

(b) Designation of drug as fast track product

(1) In general

The Secretary shall, at the request of the sponsor of a new drug, facilitate the development and expedite the review of such drug if it is intended, whether alone or in combination with one or more other drugs, for the treatment of a serious or life-threatening disease or condition, and it demonstrates the potential to address unmet medical needs for such a disease or condition, or if the Secretary designates the drug as a qualified infectious disease product under section 355f(d) of this title. (In this section, such a drug is referred to as a “fast track product”.)

(2) Request for designation

The sponsor of a new drug may request the Secretary to designate the drug as a fast track product. A request for the designation may be made concurrently with, or at any time after, submission of an application for the investigation of the drug under section 355(i) of this title or section 262(a)(3) of title 42.

(3) Designation

Within 60 calendar days after the receipt of a request under paragraph (2), the Secretary shall determine whether the drug that is the subject of the request meets the criteria described in paragraph (1). If the Secretary finds that the drug meets the criteria, the Secretary shall designate the drug as a fast track product and shall take such actions as are appropriate to expedite the development and review of the application for approval of such product.

(c) Accelerated approval of a drug for a serious or life-threatening disease or condition, including a fast track product

(1) In general

(A) Accelerated approval

The Secretary may approve an application for approval of a product for a serious or life-threatening disease or condition, including a fast track product, under section 355(c) of this title or section 262(a) of title 42 upon a determination that the product has an effect on a surrogate endpoint that is reasonably likely to predict clinical benefit, or on a clinical endpoint that can be measured earlier than irreversible morbidity or mortality, that is reasonably likely to predict an effect on irreversible morbidity or mortality or other clinical benefit, taking into account the severity, rarity, or prevalence of the condition and the availability or lack of alternative treatments. The approval described in the preceding sentence is referred to in this section as “accelerated approval”.

(B) Evidence

The evidence to support that an endpoint is reasonably likely to predict clinical benefit under subparagraph (A) may include epidemiological, pathophysiological, therapeutic, pharmacologic, or other evidence developed using biomarkers, for example, or other scientific methods or tools.

(2) Limitation

Approval of a product under this subsection may be subject to 1 or both of the following requirements:

(A) That the sponsor conduct appropriate postapproval studies to verify and describe the predicted effect on irreversible morbidity or mortality or other clinical benefit.

(B) That the sponsor submit copies of all promotional materials related to the product during the preapproval review period and, following approval and for such period thereafter as the Secretary determines to be appropriate, at least 30 days prior to dissemination of the materials.

(3) Expedited withdrawal of approval

The Secretary may withdraw approval of a product approved under accelerated approval using expedited procedures (as prescribed by the Secretary in regulations which shall include an opportunity for an informal hearing) if—

(A) the sponsor fails to conduct any required postapproval study of the drug with due diligence;

(B) a study required to verify and describe the predicted effect on irreversible morbidity or mortality or other clinical benefit of the product fails to verify and describe such effect or benefit;

(C) other evidence demonstrates that the product is not safe or effective under the conditions of use; or

(D) the sponsor disseminates false or misleading promotional materials with respect to the product.

(d) Review of incomplete applications for approval of a fast track product

(1) In general

If the Secretary determines, after preliminary evaluation of clinical data submitted by the sponsor, that a fast track product may be effective, the Secretary shall evaluate for filing, and may commence review of portions of, an application for the approval of the product before the sponsor submits a complete application. The Secretary shall commence such review only if the applicant—

(A) provides a schedule for submission of information necessary to make the application complete; and

(B) pays any fee that may be required under section 379h of this title.

(2) Exception

Any time period for review of human drug applications that has been agreed to by the Secretary and that has been set forth in goals identified in letters of the Secretary (relating to the use of fees collected under section 379h of this title to expedite the drug development process and the review of human drug applications) shall not apply to an application submitted under paragraph (1) until the date on which the application is complete.

(e) Construction

(1) Purpose

The amendments made by the Food and Drug Administration Safety and Innovation Act to this section are intended to encourage the Secretary to utilize innovative and flexible approaches to the assessment of products under accelerated approval for treatments for patients with serious or life-threatening diseases or conditions and unmet medical needs.

(2) Construction

Nothing in this section shall be construed to alter the standards of evidence under subsection (c) or (d) of section 355 of this title (including the substantial evidence standard in section 355(d) of this title) or under section 262(a) of title 42. Such sections and standards of evidence apply to the review and approval of products under this section, including whether a product is safe and effective. Nothing in this section alters the ability of the Secretary to rely on evidence that does not come from adequate and well-controlled investigations for the purpose of determining whether an endpoint is reasonably likely to predict clinical benefit as described in subsection (b)(1)(B).

(f) Awareness efforts

The Secretary shall—

(1) develop and disseminate .to physicians, patient organizations, pharmaceutical and biotechnology companies, and other appropriate persons a description of the provisions of this section applicable to breakthrough therapies, accelerated approval, and and[1] fast track products; and

(2) establish a program to encourage the development of surrogate and clinical endpoints, including biomarkers, and other scientific methods and tools that can assist the Secretary in determining whether the evidence submitted in an application is reasonably likely to predict clinical benefit for serious or life-threatening conditions for which significant unmet medical needs exist.

[1] So in original.

Subpart J—Records and Reports

§ 211.180 General requirements.

(a) Any production, control, or distribution record that is required to be maintained in compliance with this part and is specifically associated with a batch of a drug product shall be retained for at least 1 year after the expiration date of the batch or, in the case of certain OTC drug products lacking expiration dating because they meet the criteria for exemption under § 211.137, 3 years after distribution of the batch.

(b) Records shall be maintained for all components, drug product containers, closures, and labeling for at least 1 year after the expiration date or, in the case of certain OTC drug products lacking expiration dating because they meet the criteria for exemption under § 211.137, 3 years after distribution of the last lot of drug product incorporating the component or using the container, closure, or labeling.

(c) All records required under this part, or copies of such records, shall be readily available for authorized inspection during the retention period at the establishment where the activities described in such records occurred. These records or copies thereof shall be subject to photocopying or other means of reproduction as part of such inspection. Records that can be immediately retrieved from another location by computer or other electronic means shall be considered as meeting the requirements of this paragraph.

(d) Records required under this part may be retained either as original records or as true copies such as photocopies, microfilm, microfiche, or other accurate reproductions of the original records. Where reduction techniques, such as microfilming, are used, suitable reader and photocopying equipment shall be readily available.

(e) Written records required by this part shall be maintained so that data therein can be used for evaluating, at least annually, the quality standards of each drug product to determine the need for changes in drug product specifications or manufacturing or control procedures. Written procedures shall be established and followed for such evaluations and shall include provisions for:

(1) A review of a representative number of batches, whether approved or rejected, and, where applicable, records associated with the batch.

(2) A review of complaints, recalls, returned or salvaged drug products, and investigations conducted under § 211.192 for each drug product.

(f) Procedures shall be established to assure that the responsible officials of the firm, if they are not personally involved in or immediately aware of such actions, are notified in writing of any investigations conducted under §§ 211.198, 211.204, or 211.208 of these regulations, any recalls, reports of inspectional observations issued by the Food and Drug Administration, or any regulatory actions relating to good manufacturing practices brought by the Food and Drug Administration.

[43 FR 45077, Sept. 29, 1978, as amended at 60 FR 4091, Jan. 20, 1995]

§ 211.182 Equipment cleaning and use log.

A written record of major equipment cleaning, maintenance (except routine maintenance such as lubrication and adjustments), and use shall be included in individual equipment logs that show the date, time, product, and lot number of each batch processed. If equipment is dedicated to manufacture of one product, then individual equipment logs are not required, provided that lots or batches of such product follow in numerical order and are manufactured in numerical sequence. In cases where dedicated equipment is employed, the records of cleaning, maintenance, and use shall be part of the batch record. The persons performing and double-checking the cleaning and maintenance (or, if the cleaning and maintenance is performed using automated equipment under § 211.68, just the person verifying the cleaning and maintenance done by the automated equipment) shall date and sign or initial the log indicating that the work was performed. Entries in the log shall be in chronological order.

[73 FR 51933, Sept. 8, 2008]

§ 211.184 Component, drug product container, closure, and labeling records.

These records shall include the following:

(a) The identity and quantity of each shipment of each lot of components, drug product containers, closures, and labeling; the name of the supplier; the supplier's lot number(s) if known; the receiving code as specified in § 211.80; and the date of receipt. The name and location of the prime manufacturer, if different from the supplier, shall be listed if known.

(b) The results of any test or examination performed (including those performed as required by § 211.82(a), § 211.84(d), or § 211.122(a)) and the conclusions derived therefrom.

(c) An individual inventory record of each component, drug product container, and closure and, for each component, a reconciliation of the use of each lot of such component. The inventory record shall contain sufficient information to allow determination of any batch or lot of drug product associated with the use of each component, drug product container, and closure.

(d) Documentation of the examination and review of labels and labeling for conformity with established specifications in accord with §§ 211.122(c) and 211.130(c).

(e) The disposition of rejected components, drug product containers, closure, and labeling.

§ 211.186 Master production and control records.

(a) To assure uniformity from batch to batch, master production and control records for each drug product, including each batch size thereof, shall be prepared, dated, and signed (full signature, handwritten) by one person and independently checked, dated, and signed by a second person. The preparation of master production and control records shall be described in a written procedure and such written procedure shall be followed.

(b) Master production and control records shall include:

(1) The name and strength of the product and a description of the dosage form;

(2) The name and weight or measure of each active ingredient per dosage unit or per unit of weight or measure of the drug product, and a statement of the total weight or measure of any dosage unit;

(3) A complete list of components designated by names or codes sufficiently specific to indicate any special quality characteristic;

(4) An accurate statement of the weight or measure of each component, using the same weight system (metric, avoirdupois, or apothecary) for each component. Reasonable variations may be permitted, however, in the amount of components necessary for the preparation in the dosage form, provided they are justified in the master production and control records;

(5) A statement concerning any calculated excess of component;

(6) A statement of theoretical weight or measure at appropriate phases of processing;

(7) A statement of theoretical yield, including the maximum and minimum percentages of theoretical yield beyond which investigation according to § 211.192 is required;

(8) A description of the drug product containers, closures, and packaging materials, including a specimen or copy of each label and all other labeling signed and dated by the person or persons responsible for approval of such labeling;

(9) Complete manufacturing and control instructions, sampling and testing procedures, specifications, special notations, and precautions to be followed.

§ 211.188 Batch production and control records.

Batch production and control records shall be prepared for each batch of drug product produced and shall include complete information relating to the production and control of each batch. These records shall include:

(a) An accurate reproduction of the appropriate master production or control record, checked for accuracy, dated, and signed;

(b) Documentation that each significant step in the manufacture, processing, packing, or holding of the batch was accomplished, including:

(1) Dates;

(2) Identity of individual major equipment and lines used;

(3) Specific identification of each batch of component or in-process material used;

(4) Weights and measures of components used in the course of processing;

(5) In-process and laboratory control results;

(6) Inspection of the packaging and labeling area before and after use;

(7) A statement of the actual yield and a statement of the percentage of theoretical yield at appropriate phases of processing;

(8) Complete labeling control records, including specimens or copies of all labeling used;

(9) Description of drug product containers and closures;

(10) Any sampling performed;

(11) Identification of the persons performing and directly supervising or checking each significant step in the operation, or if a significant step in the operation is performed by automated equipment under § 211.68, the identification of the person checking the significant step performed by the automated equipment.

(12) Any investigation made according to § 211.192.

(13) Results of examinations made in accordance with § 211.134.

[43 FR 45077, Sept. 29, 1978, as amended at 73 FR 51933, Sept. 8, 2008]

§ 211.192 Production record review.

All drug product production and control records, including those for packaging and labeling, shall be reviewed and approved by the quality control unit to determine compliance with all established, approved written procedures before a batch is released or distributed. Any unexplained discrepancy (including a percentage of theoretical yield exceeding the maximum or minimum percentages established in master production and control records) or the failure of a batch or any of its components to meet any of its specifications shall be thoroughly investigated, whether or not the batch has already been distributed. The investigation shall extend to other batches of the same drug product and other drug products that may have been associated with the specific failure or discrepancy. A written record of the investigation shall be made and shall include the conclusions and followup.

§ 211.194 Laboratory records.

(a) Laboratory records shall include complete data derived from all tests necessary to assure compliance with established specifications and standards, including examinations and assays, as follows:

(1) A description of the sample received for testing with identification of source (that is, location from where sample was obtained), quantity, lot number or other distinctive code, date sample was taken, and date sample was received for testing.

(2) A statement of each method used in the testing of the sample. The statement shall indicate the location of data that establish that the methods used in the testing of the sample meet proper standards of accuracy and reliability as applied to the product tested. (If the method employed is in the current revision of the United States Pharmacopeia, National Formulary, AOAC INTERNATIONAL, Book of Methods,[1] or in other recognized standard references, or is detailed in an approved new drug application and the referenced method is not modified, a statement indicating the method and reference will suffice). The suitability of all testing methods used shall be verified under actual conditions of use.

(3) A statement of the weight or measure of sample used for each test, where appropriate.

(4) A complete record of all data secured in the course of each test, including all graphs, charts, and spectra from laboratory instrumentation, properly identified to show the specific component, drug product container, closure, in-process material, or drug product, and lot tested.

(5) A record of all calculations performed in connection with the test, including units of measure, conversion factors, and equivalency factors.

(6) A statement of the results of tests and how the results compare with established standards of identity,

[1] Copies may be obtained from: AOAC INTERNATIONAL, 481 North Frederick Ave., suite 500, Gaithersburg, MD 20877.

strength, quality, and purity for the component, drug product container, closure, in-process material, or drug product tested.

(7) The initials or signature of the person who performs each test and the date(s) the tests were performed.

(8) The initials or signature of a second person showing that the original records have been reviewed for accuracy, completeness, and compliance with established standards.

(b) Complete records shall be maintained of any modification of an established method employed in testing. Such records shall include the reason for the modification and data to verify that the modification produced results that are at least as accurate and reliable for the material being tested as the established method.

(c) Complete records shall be maintained of any testing and standardization of laboratory reference standards, reagents, and standard solutions.

(d) Complete records shall be maintained of the periodic calibration of laboratory instruments, apparatus, gauges, and recording devices required by § 211.160(b)(4).

(e) Complete records shall be maintained of all stability testing performed in accordance with § 211.166.

[43 FR 45077, Sept. 29, 1978, as amended at 55 FR 11577, Mar. 29, 1990; 65 FR 18889, Apr. 10, 2000; 70 FR 40880, July 15, 2005; 70 FR 67651, Nov. 8, 2005]

§ 211.196 Distribution records.

Distribution records shall contain the name and strength of the product and description of the dosage form, name and address of the consignee, date and quantity shipped, and lot or control number of the drug product. For compressed medical gas products, distribution records are not required to contain lot or control numbers.

(Approved by the Office of Management and Budget under control number 0910–0139)

[49 FR 9865, Mar. 16, 1984]

§ 211.198 Complaint files.

(a) Written procedures describing the handling of all written and oral complaints regarding a drug product shall be established and followed. Such procedures shall include provisions for review by the quality control unit, of any complaint involving the possible failure of a drug product to meet any of its specifications and, for such drug products, a determination as to the need for an investigation in accordance with § 211.192. Such procedures shall include provisions for review to determine whether the complaint represents a serious and unexpected adverse drug experience which is required to be reported to the Food and Drug Administration in accordance with §§ 310.305 and 514.80 of this chapter.

(b) A written record of each complaint shall be maintained in a file designated for drug product complaints. The file regarding such drug product complaints shall be maintained at the establishment where the drug product involved was manufactured, processed, or packed, or such file may be maintained at another facility if the written records in such files are readily available for inspection at that other facility. Written records involving a drug product shall be maintained until at least 1 year after the expiration date of the drug product, or 1 year after the date that the complaint was received, whichever is longer. In the case of certain OTC drug products lacking expiration dating because they meet the criteria for exemption under § 211.137, such written records shall be maintained for 3 years after distribution of the drug product.

(1) The written record shall include the following information, where known: the name and strength of the drug product, lot number, name of complainant, nature of complaint, and reply to complainant.

(2) Where an investigation under § 211.192 is conducted, the written record shall include the findings of the investigation and followup. The record or copy of the record of the investigation shall be maintained at the establishment where the investigation occurred in accordance with § 211.180(c).

(3) Where an investigation under § 211.192 is not conducted, the written record shall include the reason that an

will then request of the applicable new drug application holder that the correctness of the patent information or omission of patent information be confirmed. Unless the application holder withdraws or amends its patent information in response to FDA's request, the agency will not change the patent information in the list. If the new drug application holder does not change the patent information submitted to FDA, a 505(b)(2) application or an abbreviated new drug application under section 505(j) of the act submitted for a drug that is claimed by a patent for which information has been submitted must, despite any disagreement as to the correctness of the patent information, contain an appropriate certification for each listed patent.

[59 FR 50363, Oct. 3, 1994, as amended at 68 FR 36703, June 18, 2003; 69 FR 13473, Mar. 23, 2004; 74 FR 9766, Mar. 6, 2009; 74 FR 36605, July 24, 2009; 76 FR 31470, June 1, 2011]

§ 314.54 Procedure for submission of an application requiring investigations for approval of a new indication for, or other change from, a listed drug.

(a) The act does not permit approval of an abbreviated new drug application for a new indication, nor does it permit approval of other changes in a listed drug if investigations, other than bioavailability or bioequivalence studies, are essential to the approval of the change. Any person seeking approval of a drug product that represents a modification of a listed drug (e.g., a new indication or new dosage form) and for which investigations, other than bioavailability or bioequivalence studies, are essential to the approval of the changes may, except as provided in paragraph (b) of this section, submit a 505(b)(2) application. This application need contain only that information needed to support the modification(s) of the listed drug.

(1) The applicant shall submit a complete archival copy of the application that contains the following:

(i) The information required under §314.50(a), (b), (c), (d)(1), (d)(3), (e), and (g), except that §314.50(d)(1)(ii)(*c*) shall contain the proposed or actual master production record, including a description of the equipment, to be used for the manufacture of a commercial lot of the drug product.

(ii) The information required under §314.50 (d)(2), (d)(4) (if an anti-infective drug), (d)(5), (d)(6), and (f) as needed to support the safety and effectiveness of the drug product.

(iii) Identification of the listed drug for which FDA has made a finding of safety and effectiveness and on which finding the applicant relies in seeking approval of its proposed drug product by established name, if any, proprietary name, dosage form, strength, route of administration, name of listed drug's application holder, and listed drug's approved application number.

(iv) If the applicant is seeking approval only for a new indication and not for the indications approved for the listed drug on which the applicant relies, a certification so stating.

(v) Any patent information required under section 505(b)(1) of the act with respect to any patent which claims the drug for which approval is sought or a method of using such drug and to which a claim of patent infringement could reasonably be asserted if a person not licensed by the owner of the patent engaged in the manufacture, use, or sale of the drug product.

(vi) Any patent certification or statement required under section 505(b)(2) of the act with respect to any relevant patents that claim the listed drug or that claim any other drugs on which investigations relied on by the applicant for approval of the application were conducted, or that claim a use for the listed or other drug.

(vii) If the applicant believes the change for which it is seeking approval is entitled to a period of exclusivity, the information required under §314.50(j).

(2) The applicant shall submit a review copy that contains the technical sections described in §314.50(d)(1), except that §314.50(d)(1)(ii)(*c*) shall contain the proposed or actual master production record, including a description of the equipment, to be used for the manufacture of a commercial lot of the drug product, and paragraph (d)(3), and the technical sections described in paragraphs (d)(2), (d)(4), (d)(5), (d)(6), and (f) when needed to support the modification. Each of the technical

sections in the review copy is required to be separately bound with a copy of the information required under § 314.50 (a), (b), and (c) and a copy of the proposed labeling.

(3) The information required by § 314.50 (d)(2), (d)(4) (if an anti-infective drug), (d)(5), (d)(6), and (f) for the listed drug on which the applicant relies shall be satisfied by reference to the listed drug under paragraph (a)(1)(iii) of this section.

(4) The applicant shall submit a field copy of the application that contains the technical section described in § 314.50(d)(1), a copy of the information required under § 314.50(a) and (c), and certification that the field copy is a true copy of the technical section described in § 314.50(d)(1) contained in the archival and review copies of the application.

(b) An application may not be submitted under this section for a drug product whose only difference from the reference listed drug is that:

(1) The extent to which its active ingredient(s) is absorbed or otherwise made available to the site of action is less than that of the reference listed drug; or

(2) The rate at which its active ingredient(s) is absorbed or otherwise made available to the site of action is unintentionally less than that of the reference listed drug.

[57 FR 17982, Apr. 28, 1992; 57 FR 61612, Dec. 28, 1992, as amended at 58 FR 47351, Sept. 8, 1993; 59 FR 50364, Oct. 3, 1994]

§ 314.55 Pediatric use information.

(a) *Required assessment.* Except as provided in paragraphs (b), (c), and (d) of this section, each application for a new active ingredient, new indication, new dosage form, new dosing regimen, or new route of administration shall contain data that are adequate to assess the safety and effectiveness of the drug product for the claimed indications in all relevant pediatric subpopulations, and to support dosing and administration for each pediatric subpopulation for which the drug is safe and effective. Where the course of the disease and the effects of the drug are sufficiently similar in adults and pediatric patients, FDA may conclude that pediatric effectiveness can be extrapolated from adequate and well-controlled studies in adults usually supplemented with other information obtained in pediatric patients, such as pharmacokinetic studies. Studies may not be needed in each pediatric age group, if data from one age group can be extrapolated to another. Assessments of safety and effectiveness required under this section for a drug product that represents a meaningful therapeutic benefit over existing treatments for pediatric patients must be carried out using appropriate formulations for each age group(s) for which the assessment is required.

(b) *Deferred submission.* (1) FDA may, on its own initiative or at the request of an applicant, defer submission of some or all assessments of safety and effectiveness described in paragraph (a) of this section until after approval of the drug product for use in adults. Deferral may be granted if, among other reasons, the drug is ready for approval in adults before studies in pediatric patients are complete, or pediatric studies should be delayed until additional safety or effectiveness data have been collected. If an applicant requests deferred submission, the request must provide a certification from the applicant of the grounds for delaying pediatric studies, a description of the planned or ongoing studies, and evidence that the studies are being or will be conducted with due diligence and at the earliest possible time.

(2) If FDA determines that there is an adequate justification for temporarily delaying the submission of assessments of pediatric safety and effectiveness, the drug product may be approved for use in adults subject to the requirement that the applicant submit the required assessments within a specified time.

(c) *Waivers*—(1) *General.* FDA may grant a full or partial waiver of the requirements of paragraph (a) of this section on its own initiative or at the request of an applicant. A request for a waiver must provide an adequate justification.

(2) *Full waiver.* An applicant may request a waiver of the requirements of paragraph (a) of this section if the applicant certifies that:

(i) The drug product does not represent a meaningful therapeutic benefit over existing treatments for pediatric patients and is not likely to be used in a substantial number of pediatric patients;

(ii) Necessary studies are impossible or highly impractical because, e.g., the number of such patients is so small or geographically dispersed; or

(iii) There is evidence strongly suggesting that the drug product would be ineffective or unsafe in all pediatric age groups.

(3) *Partial waiver.* An applicant may request a waiver of the requirements of paragraph (a) of this section with respect to a specified pediatric age group, if the applicant certifies that:

(i) The drug product does not represent a meaningful therapeutic benefit over existing treatments for pediatric patients in that age group, and is not likely to be used in a substantial number of patients in that age group;

(ii) Necessary studies are impossible or highly impractical because, e.g., the number of patients in that age group is so small or geographically dispersed;

(iii) There is evidence strongly suggesting that the drug product would be ineffective or unsafe in that age group; or

(iv) The applicant can demonstrate that reasonable attempts to produce a pediatric formulation necessary for that age group have failed.

(4) *FDA action on waiver.* FDA shall grant a full or partial waiver, as appropriate, if the agency finds that there is a reasonable basis on which to conclude that one or more of the grounds for waiver specified in paragraphs (c)(2) or (c)(3) of this section have been met. If a waiver is granted on the ground that it is not possible to develop a pediatric formulation, the waiver will cover only those pediatric age groups requiring that formulation. If a waiver is granted because there is evidence that the product would be ineffective or unsafe in pediatric populations, this information will be included in the product's labeling.

(5) *Definition of ''meaningful therapeutic benefit''.* For purposes of this section and § 201.23 of this chapter, a drug will be considered to offer a meaningful therapeutic benefit over existing therapies if FDA estimates that:

(i) If approved, the drug would represent a significant improvement in the treatment, diagnosis, or prevention of a disease, compared to marketed products adequately labeled for that use in the relevant pediatric population. Examples of how improvement might be demonstrated include, for example, evidence of increased effectiveness in treatment, prevention, or diagnosis of disease, elimination or substantial reduction of a treatment-limiting drug reaction, documented enhancement of compliance, or evidence of safety and effectiveness in a new subpopulation; or

(ii) The drug is in a class of drugs or for an indication for which there is a need for additional therapeutic options.

(d) *Exemption for orphan drugs.* This section does not apply to any drug for an indication or indications for which orphan designation has been granted under part 316, subpart C, of this chapter.

[63 FR 66670, Dec. 2, 1998]

§ 314.60 Amendments to an unapproved application, supplement, or resubmission.

(a) FDA generally assumes that when an original application, supplement to an approved application, or resubmission of an application or supplement is submitted to the agency for review, the applicant believes that the agency can approve the application, supplement, or resubmission as submitted. However, the applicant may submit an amendment to an application that has been filed under § 314.101 but is not yet approved.

(b)(1) Submission of a major amendment to an original application, efficacy supplement, or resubmission of an application or efficacy supplement within 3 months of the end of the initial review cycle constitutes an agreement by the applicant under section 505(c) of the act to extend the initial review cycle by 3 months. (For references to a resubmission of an application or efficacy supplement in paragraph (b) of this section, the timeframe for reviewing the resubmission is the ''review cycle'' rather than the ''initial

review cycle.") FDA may instead defer review of the amendment until the subsequent review cycle. If the agency extends the initial review cycle for an original application, efficacy supplement, or resubmission under this paragraph, the division responsible for reviewing the application, supplement, or resubmission will notify the applicant of the extension. The initial review cycle for an original application, efficacy supplement, or resubmission of an application or efficacy supplement may be extended only once due to submission of a major amendment. FDA may, at its discretion, review any subsequent major amendment during the initial review cycle (as extended) or defer review until the subsequent review cycle.

(2) Submission of a major amendment to an original application, efficacy supplement, or resubmission of an application or efficacy supplement more than 3 months before the end of the initial review cycle will not extend the cycle. FDA may, at its discretion, review such an amendment during the initial review cycle or defer review until the subsequent review cycle.

(3) Submission of an amendment to an original application, efficacy supplement, or resubmission of an application or efficacy supplement that is not a major amendment will not extend the initial review cycle. FDA may, at its discretion, review such an amendment during the initial review cycle or defer review until the subsequent review cycle.

(4) Submission of a major amendment to a manufacturing supplement within 2 months of the end of the initial review cycle constitutes an agreement by the applicant under section 505(c) of the act to extend the initial review cycle by 2 months. FDA may instead defer review of the amendment until the subsequent review cycle. If the agency extends the initial review cycle for a manufacturing supplement under this paragraph, the division responsible for reviewing the supplement will notify the applicant of the extension. The initial review cycle for a manufacturing supplement may be extended only once due to submission of a major amendment. FDA may, at its discretion, review any subsequent major amendment during the initial review cycle (as extended) or defer review until the subsequent review cycle.

(5) Submission of an amendment to a supplement other than an efficacy or manufacturing supplement will not extend the initial review cycle. FDA may, at its discretion, review such an amendment during the initial review cycle or defer review until the subsequent review cycle.

(6) A major amendment may not include data to support an indication or claim that was not included in the original application, supplement, or resubmission, but it may include data to support a minor modification of an indication or claim that was included in the original application, supplement, or resubmission.

(7) When FDA defers review of an amendment until the subsequent review cycle, the agency will notify the applicant of the deferral in the complete response letter sent to the applicant under §314.110 of this part.

(c)(1) An unapproved application may not be amended if all of the following conditions apply:

(i) The unapproved application is for a drug for which a previous application has been approved and granted a period of exclusivity in accordance with section 505(c)(3)(D)(ii) of the act that has not expired;

(ii) The applicant seeks to amend the unapproved application to include a published report of an investigation that was conducted or sponsored by the applicant entitled to exclusivity for the drug;

(iii) The applicant has not obtained a right of reference to the investigation described in paragraph (c)(1)(ii) of this section; and

(iv) The report of the investigation described in paragraph (c)(1)(ii) of this section would be essential to the approval of the unapproved application.

(2) The submission of an amendment described in paragraph (c)(1) of this section will cause the unapproved application to be deemed to be withdrawn by the applicant under §314.65 on the date of receipt by FDA of the amendment. The amendment will be considered a resubmission of the application, which may not be accepted except as

provided in accordance with section 505(c)(3)(D)(ii) of the act.

(d) The applicant shall submit a field copy of each amendment to § 314.50(d)(1). The applicant shall include in its submission of each such amendment to FDA a statement certifying that a field copy of the amendment has been sent to the applicant's home FDA district office.

[50 FR 7493, Feb. 22, 1985, as amended at 57 FR 17983, Apr. 28, 1992; 58 FR 47352, Sept. 8, 1993; 63 FR 5252, Feb. 2, 1998; 69 FR 18764, Apr. 8, 2004; 73 FR 39608, July 10, 2008]

§ 314.65 Withdrawal by the applicant of an unapproved application.

An applicant may at any time withdraw an application that is not yet approved by notifying the Food and Drug Administration in writing. If, by the time it receives such notice, the agency has identified any deficiencies in the application, we will list such deficiencies in the letter we send the applicant acknowledging the withdrawal. A decision to withdraw the application is without prejudice to refiling. The agency will retain the application and will provide a copy to the applicant on request under the fee schedule in § 20.45 of FDA's public information regulations.

[50 FR 7493, Feb. 22, 1985, as amended at 68 FR 25287, May 12, 2003; 73 FR 39609, July 10, 2008]

§ 314.70 Supplements and other changes to an approved application.

(a) *Changes to an approved application.* (1)(i) Except as provided in paragraph (a)(1)(ii) of this section, the applicant must notify FDA about each change in each condition established in an approved application beyond the variations already provided for in the application. The notice is required to describe the change fully. Depending on the type of change, the applicant must notify FDA about the change in a supplement under paragraph (b) or (c) of this section or by inclusion of the information in the annual report to the application under paragraph (d) of this section.

(ii) The submission and grant of a written request for an exception or alternative under § 201.26 of this chapter satisfies the applicable requirements in paragraphs (a) through (c) of this section. However, any grant of a request for an exception or alternative under § 201.26 of this chapter must be reported as part of the annual report to the application under paragraph (d) of this section.

(2) The holder of an approved application under section 505 of the act must assess the effects of the change before distributing a drug product made with a manufacturing change.

(3) Notwithstanding the requirements of paragraphs (b) and (c) of this section, an applicant must make a change provided for in those paragraphs in accordance with a regulation or guidance that provides for a less burdensome notification of the change (for example, by submission of a supplement that does not require approval prior to distribution of the product or in an annual report).

(4) The applicant must promptly revise all promotional labeling and advertising to make it consistent with any labeling change implemented in accordance with paragraphs (b) and (c) of this section.

(5) Except for a supplement providing for a change in the labeling, the applicant must include in each supplement and amendment to a supplement providing for a change under paragraph (b) or (c) of this section a statement certifying that a field copy has been provided in accordance with § 314.440(a)(4).

(6) A supplement or annual report must include a list of all changes contained in the supplement or annual report. For supplements, this list must be provided in the cover letter.

(b) *Changes requiring supplement submission and approval prior to distribution of the product made using the change (major changes).* (1) A supplement must be submitted for any change in the drug substance, drug product, production process, quality controls, equipment, or facilities that has a substantial potential to have an adverse effect on the identity, strength, quality, purity, or potency of the drug product as these factors may relate to the safety or effectiveness of the drug product.

(2) These changes include, but are not limited to:

(i) Except those described in paragraphs (c) and (d) of this section,

changes in the qualitative or quantitative formulation of the drug product, including inactive ingredients, or in the specifications provided in the approved application;

(ii) Changes requiring completion of studies in accordance with part 320 of this chapter to demonstrate the equivalence of the drug product to the drug product as manufactured without the change or to the reference listed drug;

(iii) Changes that may affect drug substance or drug product sterility assurance, such as changes in drug substance, drug product, or component sterilization method(s) or an addition, deletion, or substitution of steps in an aseptic processing operation;

(iv) Changes in the synthesis or manufacture of the drug substance that may affect the impurity profile and/or the physical, chemical, or biological properties of the drug substance;

(v) The following labeling changes:

(A) Changes in labeling, except those described in paragraphs (c)(6)(iii), (d)(2)(ix), or (d)(2)(x) of this section;

(B) If applicable, any change to a Medication Guide required under part 208 of this chapter, except for changes in the information specified in § 208.20(b)(8)(iii) and (b)(8)(iv) of this chapter; and

(C) Any change to the information required by § 201.57(a) of this chapter, with the following exceptions that may be reported in an annual report under paragraph (d)(2)(x) of this section:

(*1*) Removal of a listed section(s) specified in § 201.57(a)(5) of this chapter; and

(*2*) Changes to the most recent revision date of the labeling as specified in § 201.57(a)(15) of this chapter.

(vi) Changes in a drug product container closure system that controls the drug product delivered to a patient or changes in the type (e.g., glass to high density polyethylene (HDPE), HDPE to polyvinyl chloride, vial to syringe) or composition (e.g., one HDPE resin to another HDPE resin) of a packaging component that may affect the impurity profile of the drug product.

(vii) Changes solely affecting a natural product, a recombinant DNA-derived protein/polypeptide, or a complex or conjugate of a drug substance with a monoclonal antibody for the following:

(A) Changes in the virus or adventitious agent removal or inactivation method(s);

(B) Changes in the source material or cell line; and

(C) Establishment of a new master cell bank or seed.

(viii) Changes to a drug product under an application that is subject to a validity assessment because of significant questions regarding the integrity of the data supporting that application.

(3) The applicant must obtain approval of a supplement from FDA prior to distribution of a drug product made using a change under paragraph (b) of this section. Except for submissions under paragraph (e) of this section, the following information must be contained in the supplement:

(i) A detailed description of the proposed change;

(ii) The drug product(s) involved;

(iii) The manufacturing site(s) or area(s) affected;

(iv) A description of the methods used and studies performed to assess the effects of the change;

(v) The data derived from such studies;

(vi) For a natural product, a recombinant DNA-derived protein/polypeptide, or a complex or conjugate of a drug substance with a monoclonal antibody, relevant validation protocols and a list of relevant standard operating procedures must be provided in addition to the requirements in paragraphs (b)(3)(iv) and (b)(3)(v) of this section; and

(vii) For sterilization process and test methodologies related to sterilization process validation, relevant validation protocols and a list of relevant standard operating procedures must be provided in addition to the requirements in paragraphs (b)(3)(iv) and (b)(3)(v) of this section.

(4) An applicant may ask FDA to expedite its review of a supplement for public health reasons or if a delay in making the change described in it would impose an extraordinary hardship on the applicant. Such a supplement and its mailing cover should be

plainly marked: "Prior Approval Supplement-Expedited Review Requested."

(c) *Changes requiring supplement submission at least 30 days prior to distribution of the drug product made using the change (moderate changes).* (1) A supplement must be submitted for any change in the drug substance, drug product, production process, quality controls, equipment, or facilities that has a moderate potential to have an adverse effect on the identity, strength, quality, purity, or potency of the drug product as these factors may relate to the safety or effectiveness of the drug product. If the supplement provides for a labeling change under paragraph (c)(6)(iii) of this section, 12 copies of the final printed labeling must be included.

(2) These changes include, but are not limited to:

(i) A change in the container closure system that does not affect the quality of the drug product, except those described in paragraphs (b) and (d) of this section; and

(ii) Changes solely affecting a natural protein, a recombinant DNA-derived protein/polypeptide or a complex or conjugate of a drug substance with a monoclonal antibody, including:

(A) An increase or decrease in production scale during finishing steps that involves different equipment; and

(B) Replacement of equipment with that of a different design that does not affect the process methodology or process operating parameters.

(iii) Relaxation of an acceptance criterion or deletion of a test to comply with an official compendium that is consistent with FDA statutory and regulatory requirements.

(3) A supplement submitted under paragraph (c)(1) of this section is required to give a full explanation of the basis for the change and identify the date on which the change is to be made. The supplement must be labeled "Supplement—Changes Being Effected in 30 Days" or, if applicable under paragraph (c)(6) of this section, "Supplement—Changes Being Effected."

(4) Pending approval of the supplement by FDA, except as provided in paragraph (c)(6) of this section, distribution of the drug product made using the change may begin not less than 30 days after receipt of the supplement by FDA. The information listed in paragraphs (b)(3)(i) through (b)(3)(vii) of this section must be contained in the supplement.

(5) The applicant must not distribute the drug product made using the change if within 30 days following FDA's receipt of the supplement, FDA informs the applicant that either:

(i) The change requires approval prior to distribution of the drug product in accordance with paragraph (b) of this section; or

(ii) Any of the information required under paragraph (c)(4) of this section is missing; the applicant must not distribute the drug product made using the change until the supplement has been amended to provide the missing information.

(6) The agency may designate a category of changes for the purpose of providing that, in the case of a change in such category, the holder of an approved application may commence distribution of the drug product involved upon receipt by the agency of a supplement for the change. These changes include, but are not limited to:

(i) Addition to a specification or changes in the methods or controls to provide increased assurance that the drug substance or drug product will have the characteristics of identity, strength, quality, purity, or potency that it purports or is represented to possess;

(ii) A change in the size and/or shape of a container for a nonsterile drug product, except for solid dosage forms, without a change in the labeled amount of drug product or from one container closure system to another;

(iii) Changes in the labeling to reflect newly acquired information, except for changes to the information required in § 201.57(a) of this chapter (which must be made under paragraph (b)(2)(v)(C) of this section), to accomplish any of the following:

(A) To add or strengthen a contraindication, warning, precaution, or adverse reaction for which the evidence of a causal association satisfies the standard for inclusion in the labeling under § 201.57(c) of this chapter;

(B) To add or strengthen a statement about drug abuse, dependence, psychological effect, or overdosage;

(C) To add or strengthen an instruction about dosage and administration that is intended to increase the safe use of the drug product;

(D) To delete false, misleading, or unsupported indications for use or claims for effectiveness; or

(E) Any labeling change normally requiring a supplement submission and approval prior to distribution of the drug product that FDA specifically requests be submitted under this provision.

(7) If the agency disapproves the supplemental application, it may order the manufacturer to cease distribution of the drug product(s) made with the manufacturing change.

(d) *Changes to be described in an annual report (minor changes).* (1) Changes in the drug substance, drug product, production process, quality controls, equipment, or facilities that have a minimal potential to have an adverse effect on the identity, strength, quality, purity, or potency of the drug product as these factors may relate to the safety or effectiveness of the drug product must be documented by the applicant in the next annual report in accordance with § 314.81(b)(2).

(2) These changes include, but are not limited to:

(i) Any change made to comply with a change to an official compendium, except a change described in paragraph (c)(2)(iii) of this section, that is consistent with FDA statutory and regulatory requirements.

(ii) The deletion or reduction of an ingredient intended to affect only the color of the drug product;

(iii) Replacement of equipment with that of the same design and operating principles except those equipment changes described in paragraph (c) of this section;

(iv) A change in the size and/or shape of a container containing the same number of dosage units for a nonsterile solid dosage form drug product, without a change from one container closure system to another;

(v) A change within the container closure system for a nonsterile drug product, based upon a showing of equivalency to the approved system under a protocol approved in the application or published in an official compendium;

(vi) An extension of an expiration dating period based upon full shelf life data on production batches obtained from a protocol approved in the application;

(vii) The addition or revision of an alternative analytical procedure that provides the same or increased assurance of the identity, strength, quality, purity, or potency of the material being tested as the analytical procedure described in the approved application, or deletion of an alternative analytical procedure;

(viii) The addition by embossing, debossing, or engraving of a code imprint to a solid oral dosage form drug product other than a modified release dosage form, or a minor change in an existing code imprint;

(ix) A change in the labeling concerning the description of the drug product or in the information about how the drug product is supplied, that does not involve a change in the dosage strength or dosage form; and

(x) An editorial or similar minor change in labeling, including a change to the information allowed by paragraphs (b)(2)(v)(C)(*1*) and (*2*) of this section.

(3) For changes under this category, the applicant is required to submit in the annual report:

(i) A statement by the holder of the approved application that the effects of the change have been assessed;

(ii) A full description of the manufacturing and controls changes, including the manufacturing site(s) or area(s) involved;

(iii) The date each change was implemented;

(iv) Data from studies and tests performed to assess the effects of the change; and,

(v) For a natural product, recombinant DNA-derived protein/polypeptide, complex or conjugate of a drug substance with a monoclonal antibody, sterilization process or test methodology related to sterilization process validation, a cross-reference to relevant validation protocols and/or standard operating procedures.

(e) *Protocols.* An applicant may submit one or more protocols describing the specific tests and studies and acceptance criteria to be achieved to demonstrate the lack of adverse effect for specified types of manufacturing changes on the identity, strength, quality, purity, and potency of the drug product as these factors may relate to the safety or effectiveness of the drug product. Any such protocols, if not included in the approved application, or changes to an approved protocol, must be submitted as a supplement requiring approval from FDA prior to distribution of a drug product produced with the manufacturing change. The supplement, if approved, may subsequently justify a reduced reporting category for the particular change because the use of the protocol for that type of change reduces the potential risk of an adverse effect.

(f) *Patent information.* The applicant must comply with the patent information requirements under section 505(c)(2) of the act.

(g) *Claimed exclusivity.* If an applicant claims exclusivity under § 314.108 upon approval of a supplement for change to its previously approved drug product, the applicant must include with its supplement the information required under § 314.50(j).

[69 FR 18764, Apr. 8, 2004, as amended at 71 FR 3997, Jan. 24, 2006; 72 FR 73600, Dec. 28, 2007; 73 FR 49609, Aug. 22, 2008]

§ 314.71 Procedures for submission of a supplement to an approved application.

(a) Only the applicant may submit a supplement to an application.

(b) All procedures and actions that apply to an application under § 314.50 also apply to supplements, except that the information required in the supplement is limited to that needed to support the change. A supplement is required to contain an archival copy and a review copy that include an application form and appropriate technical sections, samples, and labeling; except that a supplement for a change other than a change in labeling is required also to contain a field copy.

(c) All procedures and actions that apply to applications under this part, including actions by applicants and the Food and Drug Administration, also apply to supplements except as specified otherwise in this part.

[50 FR 7493, Feb. 22, 1985, as amended at 50 FR 21238, May 23, 1985; 58 FR 47352, Sept. 8, 1993; 67 FR 9586, Mar. 4, 2002; 73 FR 39609, July 10, 2008]

§ 314.72 Change in ownership of an application.

(a) An applicant may transfer ownership of its application. At the time of transfer the new and former owners are required to submit information to the Food and Drug Administration as follows:

(1) The former owner shall submit a letter or other document that states that all rights to the application have been transferred to the new owner.

(2) The new owner shall submit an application form signed by the new owner and a letter or other document containing the following:

(i) The new owner's commitment to agreements, promises, and conditions made by the former owner and contained in the application;

(ii) The date that the change in ownership is effective; and

(iii) Either a statement that the new owner has a complete copy of the approved application, including supplements and records that are required to be kept under § 314.81, or a request for a copy of the application from FDA's files. FDA will provide a copy of the application to the new owner under the fee schedule in § 20.45 of FDA's public information regulations.

(b) The new owner shall advise FDA about any change in the conditions in the approved application under § 314.70, except the new owner may advise FDA in the next annual report about a change in the drug product's label or labeling to change the product's brand or the name of its manufacturer, packer, or distributor.

[50 FR 7493, Feb. 22, 1985; 50 FR 14212, Apr. 11, 1985, as amended at 50 FR 21238, May 23, 1985; 67 FR 9586, Mar. 4, 2002; 68 FR 25287, May 12, 2003]

§ 314.80 Postmarketing reporting of adverse drug experiences.

(a) *Definitions.* The following definitions of terms apply to this section:

(7) If the applicant does not reside or have a place of business in the United States, the name and address of an agent in the United States authorized to accept service of process for the applicant.

(d) *Amendment to an abbreviated application.* If an abbreviated application is amended to include the certification described in §314.94(a)(12)(i)(A)(*4*), the applicant shall send the notice required by paragraph (a) of this section at the same time that the amendment to the abbreviated application is submitted to FDA.

(e) *Documentation of receipt of notice.* The applicant shall amend its abbreviated application to document receipt of the notice required under paragraph (a) of this section by each person provided the notice. The applicant shall include a copy of the return receipt or other similar evidence of the date the notification was received. FDA will accept as adequate documentation of the date of receipt a return receipt or a letter acknowledging receipt by the person provided the notice. An applicant may rely on another form of documentation only if FDA has agreed to such documentation in advance. A copy of the notice itself need not be submitted to the agency.

(f) *Approval.* If the requirements of this section are met, FDA will presume the notice to be complete and sufficient, and it will count the day following the date of receipt of the notice by the patent owner or its representative and by the approved application holder as the first day of the 45-day period provided for in section 505(j)(4)(B)(iii) of the act. FDA may, if the applicant provides a written statement to FDA that a later date should be used, count from such later date.

[59 FR 50366, Oct. 3, 1994, as amended at 68 FR 36705, June 18, 2003; 69 FR 11310, Mar. 10, 2004; 74 FR 9766, Mar. 6, 2009; 74 FR 36605, July 24, 2009]

§ 314.96 Amendments to an unapproved abbreviated application.

(a) *Abbreviated new drug application.* (1) An applicant may amend an abbreviated new drug application that is submitted under §314.94, but not yet approved, to revise existing information or provide additional information. Amendments containing bioequivalence studies must contain reports of all bioequivalence studies conducted by the applicant on the same drug product formulation, unless the information has previously been submitted to FDA in the abbreviated new drug application. A complete study report must be submitted for any bioequivalence study upon which the applicant relies for approval. For all other bioequivalence studies conducted on the same drug product formulation as defined in §320.1(g) of this chapter, the applicant must submit either a complete or summary report. If a summary report of a bioequivalence study is submitted and FDA determines that there may be bioequivalence issues or concerns with the product, FDA may require that the applicant submit a complete report of the bioequivalence study to FDA.

(2) Submission of an amendment containing significant data or information before the end of the initial review cycle constitutes an agreement between FDA and the applicant to extend the initial review cycle only for the time necessary to review the significant data or information and for no more than 180 days.

(b) The applicant shall submit a field copy of each amendment to §314.94(a)(9). The applicant, other than a foreign applicant, shall include in its submission of each such amendment to FDA a statement certifying that a field copy of the amendment has been sent to the applicant's home FDA district office.

[57 FR 17983, Apr. 28, 1992, as amended at 58 FR 47352, Sept. 8, 1993; 64 FR 401, Jan. 5, 1999; 73 FR 39609, July 10, 2008; 74 FR 2861, Jan. 16, 2009]

§ 314.97 Supplements and other changes to an approved abbreviated application.

The applicant shall comply with the requirements of §§314.70 and 314.71 regarding the submission of supplemental applications and other changes to an approved abbreviated application.

§ 314.98 Postmarketing reports.

(a) Except as provided in paragraph (b) of this section, each applicant having an approved abbreviated new drug

be made. *See Stickle v. Heublein, Inc.*, 716 F.2d 1550, 1564 (Fed.Cir.1983).

VI.

For the foregoing reasons, this court vacates the district court's claim construction and vacates the grant of summary judgment for non-infringement. This court remands for further proceedings. Finally, this court affirms the district court's personal jurisdiction determination.

AFFIRMED–IN–PART, VACATED–IN–PART, AND REMANDED

MOMENTA PHARMACEUTICALS, INC., Plaintiff–Appellee,

and

Sandoz, Inc., Plaintiff–Appellee,

v.

AMPHASTAR PHARMACEUTICALS, INC., International Medication Systems, Ltd., Watson Pharmaceuticals, Inc., and Watson Pharma., Inc., Defendants–Appellants.

Nos. 2012–1062, 2012–1103, 2012–1104.

United States Court of Appeals, Federal Circuit.

Aug. 3, 2012.

Background: In patent infringement litigation involving a generic version of a patented drug that prevented blood clots, the United States District Court for the District of Massachusetts, Nathaniel M. Gorton, J., —— F.Supp.2d ——, 2011 WL 5114475, denied generic manufacturers' emergency motion to dissolve or stay a preliminary injunction previously entered in the case, and manufacturers appealed.

Holding: The Court of Appeals, Moore, Circuit Judge, held that generic drug manufacturers' use of patented invention in tests conducted and submitted to Food and Drug Administration's (FDA) in order to satisfy FDA's requirements that each batch of enoxaparin that was sold commercially after FDA approval was actually the same as the brand name drug was within scope of Drug Price Competition and Patent Term Restoration Act's (Hatch–Waxman) safe harbor provision.

Vacated and remanded.

Rader, Chief Judge, filed dissenting opinion.

1. Federal Courts ⚿815

Under abuse of discretion standard of review, a grant of a preliminary injunction can be overturned by showing that the court made a clear error of judgment in weighing relevant factors or exercised its discretion based upon an error of law or clearly erroneous factual findings.

2. Patents ⚿260

Drug Price Competition and Patent Term Restoration Act's (Hatch–Waxman) safe harbor provision is broad enough to encompass submissions made pursuant to the Federal Food, Drug, and Cosmetic Act; as long as the allegedly infringing use is "for uses reasonably related" to the development and submission of that infor-

mation, it is not an act of patent infringement, regardless of where that requirement resides in the law. 35 U.S.C.A. § 271(e)(1).

3. Patents ⚿260

Generic drug manufacturers' use of patented invention in tests conducted and submitted to Food and Drug Administration's (FDA), in order to satisfy FDA's requirements that each batch of enoxaparin that was sold commercially after FDA approval was actually the same as the brand name drug, was within scope of Drug Price Competition and Patent Term Restoration Act's (Hatch–Waxman) safe harbor provision; information submitted by manufacturers was necessary both to the continued approval of the ANDA (abbreviated new drug application) and to the ability to market the generic drug, and therefore, patentee assignee did not have a reasonable likelihood of success on the merits of its patent infringement claim so as to warrant a preliminary injunction. 35 U.S.C.A. § 271(e)(1).

Patents ⚿328(2)

7,575,886. Cited.

Robert S. Frank, Jr., Choate Hall & Stewart LLP, of Boston, MA, argued for both plaintiffs-appellees. With him on the brief was Eric J. Marandett. Of counsel on the brief for plaintiff-appellee for Sandoz, Inc., was Thomas P. Steindler, McDermott, Will & Emery LLP, of Washington, DC.

Patricia A. Millett, Akin Gump Strauss Hauer & Feld LLP, of Washington, DC, argued for plaintiffs-appellants. With her on the brief were Anthony T. Pierce, Mark Mansour, Emily C. Johnson and James E. Tysse; and L. Rachel Lerman, of Los Angeles, CA.

Before RADER, Chief Judge, DYK and MOORE, Circuit Judges.

Opinion for the court filed by Circuit Judge MOORE. Dissenting opinion filed by Chief Judge RADER.

MOORE, Circuit Judge.

Amphastar Pharmaceuticals, Inc., International Medication Systems, Ltd., Watson Pharmaceuticals, Inc., and Watson Pharma., Inc. (collectively, Amphastar) appeal the district court's order denying the Emergency Motion to Dissolve or Stay the preliminary injunction entered in this case. Because the district court applied an unduly narrow interpretation of the Hatch–Waxman safe harbor, 35 U.S.C. § 271(e)(1), we vacate the grant of a preliminary injunction and remand for further proceedings consistent with this opinion.

Background

This case is a patent litigation involving a generic version of Lovenox (enoxaparin), a drug that prevents blood clots. Enoxaparin is a low molecular weight version of heparin, a naturally occurring molecule. Heparin is a polymer, known as a polysaccharide, made up of long chains of sugar molecules. Heparin is not a single defined molecule. Instead, heparin molecules have considerable diversity in (1) the length of the polysaccharide chain and (2) in the component disaccharide units and the corresponding distribution of disaccharide unit sequences in the polysaccharide chains. FDA Letter to Aventis Pharmaceuticals, Inc., July 23, 2010, FDA Docket No. FDA–2003–P–0273 (FDA Letter), J.A.

291. For example, the molecular weight of heparin molecules varies between 5,000 and 40,000 daltons. *Id.* Likewise, the disaccharide units can vary between two different uronic acid components, and each of four positions on the disaccharide unit can be modified. *Id.,* J.A. 291–92. The natural diversity inherent to heparin stems from the biosynthetic pathway used to produce the molecule. *Id.,* J.A. 292.

Enoxaparin is produced by breaking the heparin polysaccharide into smaller pieces, called oligosaccharides. Because the heparin starting material is a diverse set of molecules, enoxaparin is also made up of different chain lengths and disaccharide units corresponding to the diversity in the original mix of heparin molecules. *Id.* Additional diversity is introduced based on the way in which the heparin molecule is broken down into the low molecular weight heparin product. *Id.,* JA 292–93. Thus, unlike a typical small molecule drug like penicillin, enoxaparin is made up of a range of different molecules.

This molecular diversity raises a potential problem in light of the Food and Drug Administration's (FDA's) abbreviated new drug application (ANDA) approval process. ANDAs are typically used by generic companies to obtain approval to market a generic version of an existing drug. Unlike a new drug application (NDA), an ANDA applicant is not required to submit the same extensive clinical studies typically needed to prove the drug's safety and efficacy. Instead, the ANDA applicant must submit studies to establish that its drug is bio-equivalent to the reference drug. The ANDA must also include sufficient information to establish that the generic drug has the same active ingredients as the reference drug.

The obvious complication with using an ANDA application to gain approval for enoxaparin is that it is a mixture of a number of different low molecular weight heparin molecules. In fact, Aventis, which marketed Lovenox, asked the FDA to deny approval for a generic version of enoxaparin via an ANDA unless the applicant either (1) completely characterized enoxaparin by isolating, purifying, and sequencing each of its unique polysaccharide chains, which Aventis claimed was impossible; (2) used Aventis's manufacturing process; or (3) conducted clinical trials to prove safety and efficacy (the very type of duplicative studies the ANDA approval process was designed to avoid). FDA Letter, J.A. 286. The FDA rejected Aventis's arguments, and instead explained that the ANDA "statutory provisions do not describe the type or amount of information that an ANDA applicant must submit to demonstrate that the active ingredient in the generic drug product is the same as the active ingredient in the [reference drug]." *Id.,* J.A. 294. As a result, the FDA concluded that Congress recognized that the FDA has "broad discretion with respect to the information [it] may consider in making a finding on the 'sameness' of an active ingredient." *Id.*

Consistent with this discretion, the FDA identified five criteria, or "standards for identity," that "together provide sufficient information to conclude that generic enoxaparin has the 'same' active ingredient as Lovenox." *Id.,* J.A. 295. These criteria included, *inter alia,* "[e]quivalence in disaccharide building blocks, fragment mapping, and sequence of oligosaccharide species." *Id.* The FDA explained that such equivalence is proven by "exhaustive digestion of enoxaparin with purified heparin digesting enzymes (heparinases I, II, III) and nitrous acid, among other means, to yield the constituent disaccharide building blocks comprising enoxaparin." *Id.,* J.A.

300. These disaccharides can then potentially be "separated and quantified" by a number of techniques, including capillary electrophoresis (CE), reverse phase high performance liquid chromatography (RP–HPLC), and strong anion exchange high performance liquid chromatography (SAX–HPLC). *Id.*

The FDA also suggested the identity of the disaccharides could be determined via standard techniques, including mass spectroscopy, NMR spectroscopy, modifying reagents, or modifying enzymes. These techniques identify the nature of the constituent sugars and their substitution patterns, including the sulfation and acetylation patterns, as well as "whether the disaccharide possesses, among other structures, a ... 1, 6 anhydro ring" structure. *Id.*, J.A. 300–01. Detecting the presence of a 1, 6 anhydro ring structure is particularly important for proving equivalence because "[e]quivalence in disaccharide building blocks together with equivalence in molecular weight distribution shows that generic enoxaparin contains the 1, 6 anhydro ring structure at the reducing ends of between 15 percent and 25 percent of its poly(oligo)saccharide chains." *Id.* n. 68, J.A. 301.

Amphastar was the first company to file an ANDA for a generic version of enoxaparin. It submitted its ANDA to the FDA in March 2003, and subsequently engaged in a lengthy patent litigation with Sanofi–Aventis. Amphastar received FDA approval to market its generic enoxaparin on September 19, 2011. Despite the fact that Amphastar was the first company to file an ANDA, Momenta Pharmaceuticals, Inc. and Sandoz, Inc. (collectively Momenta), who collaborated to develop a generic enoxaparin product, were the first to bring generic enoxaparin to the market-place. Momenta received FDA approval to market enoxaparin in July 2010, more than a year before Amphastar's approval. Being the only generic version of enoxaparin has it benefits: its sales generated revenues of $260 million *per quarter*. J.A. 189. The approval of Amphastar's version of enoxaparin, and the resultant ruinous competition of another generic version of the drug, threatened this unique market position. Understandably unwilling to give up a billion dollars in yearly revenue, Momenta initiated the present litigation two days after Amphastar received final FDA approval to market its generic enoxaparin.

Momenta is the assignee of United States Patent No. 7,575,886 ('886 patent). The '886 patent generally relates "to methods for analyzing heterogeneous populations of sulfated polysaccharides, e.g. heparin [and] ... LMWH [e.g., enoxaparin.]" '886 patent col.4 ll.53–55. Claim 6 is typical. It is a method for analyzing an enoxaparin sample "for the presence or amount of a non naturally occurring sugar ... that results from a method of making enoxaparin that included β-eliminative cleavage with a benzyl ester and depolymerization." *Id.* col.64 ll.35–39. Momenta also asserted independent claims 15, which assesses the level of non-naturally occurring sugar, and 53, which allows selection of an appropriate batch. These claims are similar to claim 6. The asserted claims generally require digestion of an enoxaparin sample with a heparin degrading enzyme, followed by the use of a separation method to detect the presence of the non-naturally occurring sugar resulting from the B-eliminative cleavage. The signal corresponding to the non-naturally occurring sugar can then be used to analyze the test sample based on a comparison with a reference standard. *Id.* col.64 ll.40–57.

Momenta alleged that Amphastar infringed the '886 patent by "manufacturing generic enoxaparin for commercial sale" using the claimed methods. J.A. 58. Momenta asserted that Amphastar "included in their process for manufacturing batches of enoxaparin sodium ... a method for determining that a defined percentage of the oligosaccharide chains that make up enoxaparin include ... a non-naturally occurring sugar that includes a 1, 6–anhydro ring structure, which method infringes the '886 patent." J.A. 57. Momenta also alleged that this infringing testing was necessary because the "FDA requires a generic manufacture to include in its manufacturing process the analysis of each batch of its enoxaparin drug substance to confirm that ... [it] includes a 1, 6–anhydro ring structure." J.A. 56. Momenta moved for and received a temporary restraining order to prevent the irreparable harm of additional generic entry from Amphastar. J.A. 4. The district court subsequently granted Momenta a preliminary injunction based on its belief that Amphastar's quality control batch testing infringed the '886 patent. J.A. 30. Amphastar later filed two emergency motions for relief from the preliminary injunction, which the district court denied.

Amphastar sequentially appealed the preliminary injunction and the two denials for relief from the preliminary injunction. These three appeals were consolidated. We have jurisdiction to hear these appeals pursuant to 28 U.S.C. § 1292. After hearing oral argument in this case, we stayed the preliminary injunction. This stay, however, was not a final decision on the merits of Amphastar's appeal. We now explain why the district court incorrectly concluded that Momenta was likely to succeed on the merits of its infringement claim, and conclude that the preliminary injunction must be vacated.

Analysis

[1] "The issuance of a preliminary injunction ... is a matter of discretion for a district court. That discretion, however, is not absolute and must be reviewed in light of the equitable standards governing the issuance of injunctions." *Intel Corp. v. ULSI Sys. Tech., Inc.*, 995 F.2d 1566, 1568 (Fed.Cir.1993). To determine whether a preliminary injunction is appropriate, the district court weighs factors including "(1) whether the movant has sufficiently established a reasonable likelihood of success on the merits; (2) whether the movant would suffer irreparable harm if the injunction were not granted; (3) whether the balance of hardships tips in the movant's favor; and (4) the impact, if any, of the injunction on the public interest." *Id.* The grant of a preliminary injunction can be overturned "by showing that the court made a clear error of judgment in weighing relevant factors or exercised its discretion based upon an error of law or clearly erroneous factual findings." *Genentech, Inc. v. Novo Nordisk A/S,* 108 F.3d 1361, 1364 (Fed.Cir. 1997). As the party seeking the injunction, the burden is on Momenta to establish it is entitled to this extraordinary relief. *Id.* In order to prove a likelihood of success on the merits, Momenta must prove that Amphastar likely infringes its patent. *Id.* Conversely, if Amphastar establishes that Momenta is unlikely to succeed on its claim of infringement, a preliminary injunction is likely not appropriate. *Id.*

In its opposition to the preliminary injunction, Amphastar argued, among other things, that its testing falls within the scope of the Hatch–Waxman safe harbor, 35 U.S.C. § 271(e)(1). Section 271(e)(1) indicates that "[i]t shall not be an act of infringement to ... use ... a patented

invention ... solely for uses reasonably related to the development and submission of information under a Federal law which regulates the manufacture, use, or sale of drugs....' The district court found that "the alleged infringing activity involves the use of plaintiffs' patented quality control testing methods on each commercial batch of enoxaparin that will be sold after FDA approval." J.A. 31; *see also* J.A. 56 (Momenta's complaint alleging that the "FDA requires" the testing). While acknowledging that Amphastar's use of the patented method was for the purpose of developing information to submit to the FDA, the district court nevertheless concluded that the safe harbor does not apply to Amphastar's testing: "although the safe harbor provision permits otherwise infringing activity that is conducted to obtain regulatory approval of a product, it does not permit a generic manufacturer to continue in that otherwise infringing activity after obtaining such approval." J.A. 23. In reaching this conclusion, the district court focused primarily on the legislative history of the safe harbor, as quoted in one of our prior cases, *Classen Immunotherapies, Inc. v. Biogen IDEC,* 659 F.3d 1057 (Fed. Cir.2011). J.A. 23.

On appeal, Amphastar argues that the district court took an unduly restrictive view of the safe harbor, and that its activities fall within the plain language of 35 U.S.C. § 271(e)(1). Momenta counters that the district court correctly held that the safe harbor does not apply to Amphastar's testing for two reasons. First, Momenta argues that the safe harbor does not apply to post-approval activity: "In *Classen,* this court squarely held that '[t]he [safe harbor] does not apply to information that may be routinely reported to the FDA long after marketing approval has been obtained.' " Appellee's Br. at 43 (quoting *Classen,* 659 F.3d at 1070, alterations made by Momenta). Because Amphastar's batch testing is carried out as a condition for the post-FDA approval sale of enoxaparin, Momenta argues it falls outside the scope of the safe harbor.

Second, despite its allegations and concessions, Momenta asserts the safe harbor does not apply because "the FDA does not require the use of the particular procedure that is claimed in the '886 patent." *Id.* at 41. Instead, Momenta claims that the FDA's interpretation of its statutory mandate in its letter response to Aventis's petition, J.A. 300–01, allows a variety of testing methods to be used to establish equivalence, both for the submission of an ANDA and for the undisputedly required batch testing. Appellee's Br. at 41. Momenta argues that the availability of other acceptable testing methods means that Amphastar's alleged use of the patented method is not required by the FDA, and is therefore outside of the safe harbor provision.

The parties thus present us with conflicting views about the scope of the safe harbor. If Amphastar is correct that its post-approval activities actually fall within the scope of 35 U.S.C. § 271(e)(1), Momenta is unlikely to succeed on its claim of infringement and the preliminary injunction is likely inappropriate. *Genentech,* 108 F.3d at 1364. In order to determine whether the preliminary injunction was appropriate in this case, we must first ascertain the scope of the Hatch–Waxman safe harbor provision, 35 U.S.C. § 271(e)(1).

I.

"[A]ll statutory construction cases ... begin with the language of the statute." *Barnhart v. Sigmon Coal Co.,* 534 U.S.

438, 450, 122 S.Ct. 941, 151 L.Ed.2d 908 (2002). The "first step in interpreting a statute is to determine whether the language at issue has a plain and unambiguous meaning with regard to the particular dispute in the case." *Robinson v. Shell Oil Co.*, 519 U.S. 337, 340, 117 S.Ct. 843, 136 L.Ed.2d 808 (1997). If the language of the statute is unambiguous, there is no second step: "Our inquiry must cease if the statutory language is unambiguous and 'the statutory scheme is coherent and consistent.'" *Id.* (quoting *United States v. Ron Pair Enters., Inc.*, 489 U.S. 235, 240, 109 S.Ct. 1026, 103 L.Ed.2d 290 (1989)). Whether the text of a statute is plain or ambiguous "is determined by reference to the language itself, the specific context in which the language is used, and the broader context of the statute as a whole." *Id.* at 341, 117 S.Ct. 843.

The Drug Price Competition and Patent Term Restoration Act (Hatch–Waxman Act), Public Law No. 98–417 (1984) (codified in relevant part at 35 U.S.C. § 271(e)) set up a statutory system to "balance the need to stimulate innovation against the goal of furthering the public interest." H.R. Rep. 98–857, pt. 2, at 2714 (Aug. 1, 1984), 1984 U.S.C.C.A.N. 2686, 2714. This balance is embodied, in part, in the "safe harbor" provision of 35 U.S.C. § 271(e)(1), which provides (with emphasis added) that:

> It shall not be an act of infringement to make, use, offer to sell, or sell within the United States or import into the United States a patented invention (other than a new animal drug or veterinary biological product (as those terms are used in the Federal Food, Drug, and Cosmetic Act and the Act of March 4, 1913) which is primarily manufactured using recombinant DNA, recombinant RNA, hybridoma technology, or other processes involving site specific genetic manipulation techniques) *solely for uses reasonably related to the development and submission of information under a Federal law* which regulates the manufacture, use, or sale of drugs or veterinary biological products.

Congress could not have been clearer in its choice of words: as long as the use of the patented invention is solely for uses "reasonably related" to developing and submitting information pursuant to "a Federal law" regulating the manufacture, use, or sale of drugs, it is not "an act of infringement."

[2] Although the Hatch–Waxman safe harbor provision was enacted in the context of the then-novel ANDA approval process, 35 U.S.C. § 271(e)(1) does not reference the portion of the Federal Food, Drug, and Cosmetic Act describing the ANDA requirements, e.g., 21 U.S.C. § 355(j). Instead, Congress used more flexible and expansive language to define the scope of § 271(e)(1), referring generally to "the development and submission of information under a Federal law which regulates the manufacture, use, or sale of drugs." This broad language unambiguously applies to submissions under any federal law, providing that the law "regulates the manufacture, use, or sale of drugs." Limiting the scope of 35 U.S.C. § 271(e)(1) to just the submission of information pursuant to the Federal Food, Drug, and Cosmetic Act generally, or to the ANDA provision of the Federal Food, Drug, and Cosmetic Act in specific, would read words into the statute in violation of the express language chosen by Congress.

This interpretation is also consistent with the rest of the statutory scheme. When Congress wanted to impose a limita-

tion based on the Federal Food, Drug, and Cosmetic Act, it expressly referenced the Act. For example, in the safe harbor provision, Congress excluded "a new animal drug or veterinary biological product (as those terms are used in the *Federal Food, Drug, and Cosmetic Act* and the Act of March 4, 1913)" made using certain genetic techniques. 35 U.S.C. § 271(e)(1) (emphasis added). Likewise, when Congress wanted to limit the statute to just a certain kind of submission, for example the submission of an ANDA application under 21 U.S.C. § 355(j), it specifically referenced the statutory section governing those submissions. For example, in the subsection immediately following the safe harbor, Congress defined as an act of infringement the submission of "an application under section 505(j) of the Federal Food, Drug, and Cosmetic Act [codified at 21 U.S.C. § 355(j)] or described in section 505(b)(2) of such Act for a drug claimed in a patent or the use of which is claimed in a patent." 35 U.S.C. § 271(e)(2)(A).

Unlike the closely related infringement provision, 35 U.S.C. § 271(e)(2), Congress did not link the safe harbor to the submission of an application for approval under the Federal Food, Drug, and Cosmetic Act. *Compare* 35 U.S.C. § 271(e)(1) (not an act of infringement when used for "the development and submission of information under a Federal law") *with* 35 U.S.C. § 271(e)(2)(A) (it is an act of infringement to submit "an application under section 505(j) of the Federal Food, Drug, and Cosmetic Act or described in section 505(b)(2) of such Act"). We cannot change the statutory language. We will not import the limitation of § 271(e)(2) into § 271(e)(1). "[O]ur obligation is to take statutes as we find them." *Diamond v. Chakrabarty,* 447 U.S. 303, 315, 100 S.Ct. 2204, 65 L.Ed.2d 144 (1980); *see also, e.g., Reiter v. Sonotone Corp.,* 442 U.S. 330, 344, 99 S.Ct. 2326, 60 L.Ed.2d 931 (1979) ("We must take the statute as we find it."). The statute here applies to *any* use of a patented invention as long as the use is "reasonably related to the development and submission of information under a Federal law which regulates the manufacture, use, or sale of drugs . . . ." 35 U.S.C. § 271(e)(1).

In light of these provisions, the only coherent and consistent interpretation of "a Federal law which regulates the manufacture, use, or sale of drugs" is that it must be broad enough to encompass submissions made pursuant to the Federal Food, Drug, and Cosmetic Act. Since there is no ambiguity in the language used by Congress in 35 U.S.C. § 271(e)(1), our inquiry into the scope of the safe harbor is complete. *Robinson,* 519 U.S. at 340, 117 S.Ct. 843. When the intent of Congress is expressed so clearly and consistently throughout the statute, there is neither the need nor the occasion to refer to the legislative history. *Id.* The scope of the Hatch–Waxman safe harbor does not stop at activities reasonably related to development of information submitted in an ANDA. Instead, the safe harbor applies "to the development and submission of information under a Federal law which regulates the manufacture, use, or sale of drugs or veterinary biological products." As long as the allegedly infringing use is "for uses reasonably related" to the development and submission of that information it is not an act of infringement, regardless of where that requirement resides in the law.

This analysis is not groundbreaking: the Supreme Court came to essentially the same conclusion in 1990. In *Eli Lilly & Co. v. Medtronic, Inc.,* the Court explained that "the phrase 'a Federal law which regulates the manufacture, use, or sale of

drugs' more naturally summons up the image of *an entire statutory scheme of regulation*," and not just a particular provision of the law. 496 U.S. 661, 666, 110 S.Ct. 2683, 110 L.Ed.2d 605 (1990) (emphasis added). Although the legislative history of the safe harbor only mentioned drugs, *id.* at 669 n. 2, 110 S.Ct. 2683, the Court nevertheless concluded that the safe harbor also extended to medical devices, which were also part of "a Federal law which regulates the manufacture, use or sale of drugs," namely the Federal Food, Drug, and Cosmetic Act, *id.* at 674, 110 S.Ct. 2683.

The Court later reaffirmed this expansive view, explaining: "we think it apparent from the statutory text that § 271(e)(1)'s exemption from infringement extends to all uses of patented inventions that are reasonably related to the development and submission of *any* information under the FDCA [(Food, Drug, and Cosmetic Act)]." *Merck KGaA v. Integra Lifesciences I, Ltd.*, 545 U.S. 193, 202, 125 S.Ct. 2372, 162 L.Ed.2d 160 (2005) (citing *Eli Lilly,* 496 U.S. at 665–69, 110 S.Ct. 2683). *Merck KGaA* expressly rejected the notion that the safe harbor only applies to information developed during a clinical trial. 545 U.S. at 202 n. 6, 125 S.Ct. 2372. Instead, "the statutory text makes clear that it provides a wide berth for the use of patented drugs in *activities related to the federal regulatory process*." *Id.* at 202, 125 S.Ct. 2372 (emphasis added). In light of the unqualified exemption for uses reasonably related to the development and submission of information, "[t]here is simply no room in the statute for excluding certain information from the exemption on the basis of the phase of research in which it is developed or *the particular submission in which it could be included*." *Id.* (emphasis added). The use of the word "under" in the statute is expansive. *Caraco Pharm. Labs., Ltd. v. Novo Nordisk A/S,* 566 U.S. ——, 132 S.Ct. 1670, 1683–84, 182 L.Ed.2d 678 (2012). "Under a federal law" extends beyond just the "most barebones information" required by the FDA, and instead encompasses all "materials the FDA demands in the regulatory process." *Id.*

While it is clear that the safe harbor applies to a broad set of "activities related to the federal regulatory process," *Merck KGaA,* 545 U.S. at 202, 125 S.Ct. 2372, there is an important limitation: the use must be "for uses reasonably related to the development and submission of information," 35 U.S.C. § 271(e)(1). "Reasonably related," however, does not mean that the use of the patented invention must necessarily result in submission of information to the FDA: "Congress did not limit § 271(e)(1)'s safe harbor to the development of information for inclusion in a submission to the FDA; nor did it create an exemption applicable only to the research relevant to filing an ANDA for approval of a generic drug." *Merck KGaA,* 545 U.S. at 206, 125 S.Ct. 2372. Instead, the Court explained that the safe harbor "exempted from infringement *all* uses of patented compounds 'reasonably related' to the process of developing information for submission under *any* federal law regulating the manufacture, use, or distribution of drugs." *Id.* (emphasis in original). Thus, the Court explicitly rejected the notion that § 271(e)(1) was limited "to the activities necessary to seek approval of a generic drug." *Id.* As long as the accused infringer "has a reasonable basis for believing" that use of the patented invention might yield information that "would be appropriate to include in a submission to the FDA, that use is 'reasonably related' to the 'development and submis-

sion of information under ... Federal law.' " *Id.* at 207, 125 S.Ct. 2372.

II.

[3] At the outset we are met with the contention that the information in question was not "submitted" to the FDA, *see* 35 U.S.C. § 271(e)(1) ("... solely for uses reasonably related to the development and *submission* of information ..."), but rather was retained by the ANDA holder. We do not agree. Amphastar, as a generic drug manufacturer under an ANDA, cannot sell a batch of enoxaparin unless it has established that its strength and quality is consistent with the standards set forth in the relevant official compendium. *See* 21 U.S.C. §§ 331(a), 351(b). FDA regulations require that all records associated with a produced batch of drugs, including these batch records, "be retained for at least 1 year after the expiration date of the batch." 21 C.F.R. § 211.180(a). These records "shall be readily available for authorized inspection" by the FDA at any time. 21 C.F.R. § 211.180(c). We think that the requirement to maintain records for FDA inspection satisfies the requirement that the uses be reasonably related to the development and submission of information to the FDA. It is not disputed by the parties that these records are produced in order to develop and submit to the FDA proof that the Amphastar products comply with a Federal law. The fact that the FDA does not in most cases actually inspect the records does not change the fact that they are for the "development and submission of information under a Federal law." 35 U.S.C. § 271(e)(1); *cf. Merck KGaA,* 545 U.S. at 207, 125 S.Ct. 2372 (holding that uses which are not ultimately included in a submission to the FDA are nonetheless exempted by the safe harbor). Thus, we consider this information "submitted" for purposes of the statute. We turn then to the question of whether these submissions are within the safe harbor.

In *Merck KGaA v. Integra Lifesciences I, Ltd.*, 545 U.S. 193, 125 S.Ct. 2372, 162 L.Ed.2d 160 (2005), the Supreme Court held that uses of patented inventions in preclinical research, the results of which are not ultimately included in a submission to the FDA, are nevertheless exempted from infringement by the safe harbor provision. *Id.* at 208, 125 S.Ct. 2372. The Court explained that

> Congress did not limit § 271(e)(1)'s safe harbor to the development of information for inclusion in a submission to the FDA; nor did it create an exemption applicable only to the research relevant to filing an ANDA for approval of a generic drug. Rather, it exempted from infringement *all* uses of patented compounds "reasonably related" to the process of developing information for submission under *any* federal law regulating the manufacture, use, or distribution of drugs.

Id. at 206, 125 S.Ct. 2372. Thus, it was not an act of infringement to use patented compounds in preclinical studies which were not ultimately submitted to the FDA where "there [was] a reasonable basis for believing that the experiments [would] produce the types of information that are relevant to an IND or NDA." *Id.* at 208, 125 S.Ct. 2372.

However, in *Classen Immunotherapies, Inc. v. Biogen IDEC,* 659 F.3d 1057, 1070 (Fed.Cir.2011), we held that § 271(e)(1) "does not apply to information that may be routinely reported to the FDA, long after marketing approval has been ob-

tained." At issue in *Classen* were studies to evaluate the association between the timing of childhood vaccinations and the risk of developing certain immune-mediated disorders. The studies themselves were not mandated by the FDA, but any vaccine license holder was required to report to the FDA "adverse experience information," such as adverse side effects, it acquired as a result of vaccine studies. *See* 21 C.F.R. § 600.80. We found that the studies conducted by the vaccine license holder according to patented methods were not insulated by the safe harbor because the studies did not facilitate marketing a generic drug by "expedit[ing] development of information for regulatory approval." *Classen*, 659 F.3d at 1070. We, of course, are bound by the *Classen* decision unless it is overruled en banc or by the Supreme Court. Accordingly, the scope of the safe harbor provision does not extend to "information that may be routinely reported to the FDA, long after marketing approval has been obtained."

This case, however, fits well within *Classen* because the information submitted is necessary both to the continued approval of the ANDA and to the ability to market the generic drug. Here, the submissions are not "routine submissions" to the FDA, but instead are submissions that are required to maintain FDA approval. Amphastar is required to conduct a laboratory determination of identity and strength of the active ingredient for each batch of enoxaparin. *See* 21 C.F.R. § 211.165(a). This test must be done according to the patented methods described in an official compendium, in this case the United States Pharmacopeia (USP). *See* 21 U.S.C. § 351(b) (Any "determination as to strength, quality, or purity shall be made in accordance with the tests or methods of assay set forth in such compendium."). Moreover, as described above, FDA regulations require that all such batch records "be retained for at least 1 year after the expiration date of the batch," 21 C.F.R. § 211.180(a), and that such records "shall be readily available for authorized inspection" by the FDA at any time, 21 C.F.R. § 211.180(c); *see also* 21 C.F.R. §§ 211.186, 211.188, 211.194 (requiring "master production and control records," "batch production and control records," and "laboratory records"). Failure to comply with these requirements could result in suspension or revocation of Amphastar's ANDA approval to market the drug. *See* 21 U.S.C. §§ 335a(g), 355(e). Furthermore, such testing is "a condition for [the drug's] approval and release" into commerce, 21 C.F.R. § 211.165(d), thus acting as a predicate to the ability to market the ANDA-approved drug to the public.

The submissions to the FDA in this case are anything but "routine"—they implicate Amphastar's very ability to continue its FDA approval for its ANDA and to continue manufacturing and marketing enoxaparin under its ANDA. We also note that, unlike in *Classen* where the patented studies performed were not mandated by the FDA, the information here is not generated voluntarily by the manufacturer but is generated by FDA requirements the manufacturer is obligated under penalty of law to follow. Under such circumstances, the information can be said to have been gathered solely for submission to the FDA and not, as in *Classen*, primarily for non-FDA purposes. While Momenta urges us to adopt the pre-/post-approval distinction used by the district court, we cannot: *Classen* did not turn on this artificial distinction, and the plain language of the statute is not restricted to pre-approval

activities.[1] We therefore hold that post-approval studies that are "reasonably related to the development and submission of information under a Federal law which regulates the manufacture, use, or sale of drugs" fall within the scope of the § 271(e)(1) safe harbor.

In this case, Momenta concedes that Amphastar's tests "are conducted *in order to satisfy the FDA's requirements* that each batch of enoxaparin that is sold commercially after FDA approval is actually the same as the brand name drug." Appellee's Br. at 40–41 (emphasis added); *see also* J.A. 56 (allegation that the "FDA requires" the accused testing). Under a proper construction of 35 U.S.C. § 271(e)(1), the fact that Amphastar's testing is carried out to "satisfy the FDA's requirements" means it falls within the scope of the safe harbor, even though the activity is carried out after approval. Unlike *Classen*, where the allegedly infringing activity "may" have eventually led to an FDA submission, there is no dispute in this case that Amphastar's allegedly infringing activities are carried out to "satisfy the FDA's requirements." The district court's interpretation of § 271(e)(1) was erroneous. Under the correct construction, Momenta cannot establish a likelihood of success on infringement and the preliminary injunction must be vacated. *Genentech, Inc.*, 108 F.3d at 1364.

Momenta also argues that even if 35 U.S.C. § 271(e)(1) extends to post-approval activities, Amphastar's testing is not protected because there are FDA endorsed non-infringing alternatives available. The safe harbor, however, does not mandate the use of a noninfringing alternative when one exists. The only limitation in the safe harbor is that the use must be "reasonably related to the development and submission of information" pursuant to a federal law regulating the "manufacture, use, or sale of drugs or veterinary biological products." 35 U.S.C. § 271(e)(1). The safe harbor's protection is not limited to the dire situation where the patented invention is the only way to develop and submit the information. Instead, the safe harbor expressly allows the submitter the freedom to use an otherwise patented means to develop the necessary information demanded by the "Federal law." This makes good sense because it eliminates liability for infringement when that act of infringement is, in effect, required by the federal government as part of the continuing safety and efficacy monitoring of an approved drug. It also avoids the situation here, where a drug has received approval, but is nevertheless kept from the market based on an FDA mandated testing requirement.

Momenta's interpretation is predicated upon the incorrect assumption that "solely" in the context of 35 U.S.C. § 271(e)(1) means that the patented invention must be the "sole" means of providing the information for the safe harbor to apply. This is not the language of the statute: under 35 U.S.C. § 271(e)(1), as long as the use is "reasonably related to the development and submission of information" under a relevant statute, it is not an act of infringement. "Solely" modifies "uses reasonably

1. We are puzzled by the dissent's claim that the use of the words ''solely'' and ''submitted'' require us to limit the statute to pre-approval activities. This is not the plain meaning of those words. For example, if the FDA required post-approval testing with subsequent submission of those test results, those test results were clearly generated ''solely'' for an FDA submission and equally clearly were ''submitted'' to the agency. ''Solely'' and ''submitted'' in no manner limit § 271(e)(1) to ''pre-approval testing.''

related to the development and submission of information," but does not place any other restriction on when the patented invention may be used without infringing. As long as the use of the patented invention is done to generate information that will be submitted pursuant to a relevant federal law, that use falls within the safe harbor. *Merck KGaA,* 545 U.S. at 205–206, 125 S.Ct. 2372. Momenta is therefore incorrect that the possibility that the FDA would accept the use of other, non-patented, testing methods for the development and submission of information precludes Amphastar from relying on the safe harbor in this case.[2]

Even if Momenta's strained reading of the statute was supportable, Amphastar's allegedly infringing activities are clearly carried out according to the dictates of the Federal Food, Drug, and Cosmetic Act. Under the Act, Amphastar is prohibited from selling a drug if it is adulterated. 21 U.S.C. § 331(a). A drug is adulterated if it purports to be a drug listed in an official compendium, for example the USP, but in actuality differs in composition. 21 U.S.C. § 351(b); *see also* 21 U.S.C. § 321(j) (defining "official compendium"). In order to demonstrate that a drug is not adulterated, testing must be carried out pursuant to the methods articulated in the compendium, in this case the USP. *See* 21 U.S.C. § 351(b) (Any "determination as to strength, quality, or purity shall be made in accordance with the tests or methods of assay set forth in such compendium."). "For each batch of drug product, there shall be appropriate laboratory determination of ... the identity and strength of each active ingredient...." 21 C.F.R. § 211.165(a). FDA regulations characterize this testing as "a condition for [the drug's] approval and release" into commerce. 21 C.F.R. § 211.165(d). The FDA also mandates maintenance of appropriate records related to this type of testing. *See* 21 C.F.R. § 211.180(a) (production, control, and distribution records associated with a batch of drug must be retained for at least one year after the expiration date of the batch); *see also* 21 C.F.R. §§ 211.186, 211.188, 211.194 (requiring "master production and control records," "batch production and control records," and "laboratory records").

The USP entry for enoxaparin, the drug at issue in this litigation, states: "About 20 percent of the materials contain a 1, 6–anhydro derivative on the reducing end of the chain, the range being between 15 and 25 percent." J.A. 365 (USP Revision Bulletin, Official December 1, 2008). Thus, in order to be "enoxaparin" as defined in the

2. Although the parties do not argue that FDA-mandated quality control testing during manufacturing is not done "solely" for purposes of developing and submitting information to the FDA, the dissent suggests that because Amphastar uses the patented method while manufacturing a product to sell in commerce its infringing activity does not meet the "solely" limitation in the statute. This is not a tenable reading of the statute, and is indeed contrary to precedent. The Supreme Court cases interpreting the safe harbor make clear that the safe harbor is not limited to acts which only produce information for the FDA but protects *all* acts, even interim research steps and acts that might produce other useful data, "as long as there is a reasonable basis for believing that the [act] will produce the types of information that are relevant to [a submission to the FDA]." *Merck,* 545 U.S. at 208, 125 S.Ct. 2372. We have interpreted this language of the safe harbor to allow alleged infringers to use "data from tests for more than FDA approval," such as for fund raising and other business purposes. *Abtox, Inc. v. Exitron Corp.,* 122 F.3d 1019, 1030 (Fed.Cir.1997) (holding that the alleged infringer's "intent or alternate uses [of test data] are irrelevant to its qualification to invoke the section 271(e)(1) shield").

USP entry, the marketed drug product must contain between 15 and 25 percent of the 1, 6–anhydro derivative. *Id.; see also* 21 U.S.C. § 351(b) (drug adulterated if purports to be a drug in an official compendium but its strength, quality, or purity differs from the standard set forth in the compendium). The USP also includes a specific test for the 1, 6–anhydro derivative, which "involves HPLC analysis of a depolymerized enoxaparin sodium solution by a mixture of heparinases." J.A. 369 (USP Method <207>). As the district court explained: "Claims 6, 16, and 53 of the '886 patent describe how to analyze a sample of enoxaparin to ensure its conformity to the USP Monograph standard." J.A. 8. Amphastar is required by the FDA to use this test in order to ensure its enoxaparin is not adulterated. 21 U.S.C. § 351(b). This testing, which generates information for submission pursuant to the Food, Drug, and Cosmetic Act, therefore falls squarely within the scope of the safe harbor.

Finally, the dissent suggests that we must reject any disequilibrium between sections 201 and 202 of the Hatch–Waxman Act, that is, the safe harbor should not be available unless a patent term extension is also available. Dissenting Op. at 1370–71. This is not correct. The Supreme Court in *Eli Lilly* noted that equilibrium was not always achieved. *See Eli Lilly,* 496 U.S. at 671–72, 110 S.Ct. 2683. We too have rejected this strict interpretation of the safe harbor, explaining that "statutory symmetry is preferable but not required." *Abtox,* 122 F.3d at 1029 (holding that Class II medical devices, which are not subject to a "rigorous premarket approval process" and thus cannot receive patent term extensions, are nonetheless covered by the safe harbor).

III.

Under the correct interpretation of 35 U.S.C. § 271(e)(1), Momenta's admission that Amphastar's testing is carried out to "satisfy the FDA's requirements," Appellee's Brief at 40–41, makes it unlikely that Momenta will succeed on the merits of its infringement claim. The district court's findings with respect to the irreparable harm, balance of the hardships, and public interest factors were all, to some extent, predicated on its erroneous conclusion that Momenta's patent was likely infringed by Amphastar's product. *See* J.A. 24 (applying a presumption of irreparable harm in view of Momenta's "showing of infringement and validity"); J.A. 29 (explaining that in light of the "showing of likelihood of success on the merits, the balance of hardship tips in [Momenta's] favor"); J.A. 30 (public interest favors protection of patent rights secured by valid patents). Because Momenta has not established a likelihood of success on its claim of infringement, the preliminary injunction must be vacated.

On remand, the district court may want to consider whether Momenta's admission that Amphastar's use of the patented invention is to "satisfy the FDA's requirements" makes this case amenable to summary judgment of non-infringement in favor of Amphastar. Because the safe harbor issue is dispositive, we need not reach the other arguments on appeal.

VACATED AND REMANDED.

COSTS

Costs to Appellants.

RADER, Chief Judge, dissenting.

By definition, a patent defines a right to exclude. Consistent with property principles, an infringer of a valid patent is an unlawful trespasser. The remedy for tres-

passing, in this area of property law as well as others, is removal of the trespasser. Indeed even the Constitution acknowledges the patent owner's right to exclude trespassers. U.S. Const. art. I, § 8, cl. 8. Thus, exceptions to the traditional property remedy amount to a get-out-of-jail-free card for the trespasser. Accordingly, such exceptions must occur only sparingly with awareness that this license allows the wrongdoer free reign to continue trespassing.

The public readily applauds the role of patents in the development and delivery to the marketplace of life-saving drugs or modern technology products like smartphones. At the same time, many incremental advances contribute to these monumental advances or, as in this case, enhance their delivery to the public. These incremental inventions also represent difficult and expensive advances in technology. For example, in this case, Amphastar had a strong incentive to invent this patented manufacturing method. As the first-filer, it would have obtained 180 days of market exclusivity as the only seller of the generic drug—a right worth $260 million per quarter. Nevertheless, Amphastar could not make that invention. Instead, the patentee Momenta made the investment, did the research, and engineered the new method disclosed in the '886 patent.

At that point, Amphastar stepped in and took Momenta's patented invention without permission and used it to manufacture each commercial batch it sells on the market. Indeed Amphastar continues to trespass and promises to trespass for years to come. In fact, as the court repeatedly acknowledges, Amphastar is only able to compete with Momenta by taking its patented invention. Amphastar has not developed its own method, but instead delights in trespassing and refuses to pay a reasonable royalty to make the trespass lawful.

This court would allow this arrogance to continue by expanding the limited reach of 35 U.S.C. § 271(e)(1). This expansion of the law circumvents the purpose of the law and ignores the binding precedent of *Classen Immunotherapies, Inc. v. Biogen IDEC,* 659 F.3d 1057 (Fed.Cir.2011). Sadly this result will render worthless manufacturing test method patents. Accordingly, I must respectfully dissent.

I.

The Supreme Court has observed that the text alone of § 271(e)(1) can be "not plainly comprehensible." *Eli Lilly & Co. v. Medtronic, Inc.,* 496 U.S. 661, 669, 110 S.Ct. 2683, 110 L.Ed.2d 605 (1990). The purpose of this text, which ought to inform its application, however, is evident from the legislative history. The legislative history of § 271(e)(1) includes more than 2 House reports, 25 statements and letters, and many pages of Congressional testimony. A review of this extensive material shows that section 202 of the Hatch–Waxman Act, enacted as § 271(e)(1), had the sole purpose of overruling this court's holding in *Roche Products, Inc. v. Bolar Pharmaceutical Co.,* 733 F.2d 858 (Fed. Cir.1984). In particular, § 271(e)(1) applied only in limited situations, namely pre-approval experiments to obtain FDA approval:

> The purpose of 271(e)(1) and (2) is to establish that **experimentation** with a patented drug product, when the purpose is to prepare for commercial activity which will begin after a valid patent expires, is not a patent infringement. Since the Committee's Subcommittee on

> Health and the Environment began consideration of this bill, the Court of Appeals for the Federal Circuit held that this type of **experimentation** is infringement. In *Roche Products, Inc. v. Bolar Pharmaceutical Co.,* 733 F.2d 858 (Fed. Cir.1984), the Court of Appeals for the Federal Circuit held that the **experimental use** of a drug product prior to the expiration date of a patent claiming that drug product constitutes patent infringement, even though **the only purpose of the experiments is to seek FDA approval** for the commercial sale of the drug after the patent expires. It is the Committee's view that **experimental activity** does not have any adverse economic impact on the patent owner's exclusivity during the life of a patent, but prevention of such activity would extend the patent owner's commercial exclusivity beyond the patent expiration date.

H.R.Rep. No. 98–857, pt. 1, at 45–46 (1984), 1984 U.S.C.C.A.N. 2647, 2678–2679 (emphases added).

> The provisions of section 202 of the bill have the net effect of reversing the holding of the court in *Roche Products, Inc. v. Bolar Pharmaceutical Co.,* 733 F.2d 858 (Fed.Cir.1984).

H.R.Rep. No. 98–857, pt. 2, at 27 (1984), 1984 U.S.C.C.A.N. 2686 at 2711. *See also Innovation and Patent Law Reform: Hearing on H.R. 3605 Before the Subcomm. on Courts, Civil Liberties and the Admin. of Justice of the H. Comm. on the Judiciary,* 98th Cong. 742 (1984) (statement of Laurence H. Tribe, Professor of Law, Harvard Law School) ("Section 202, the thrust of which is to overturn *Roche v. Bolar* legislatively"); *Innovation and Patent Law Reform: Hearing on H.R. 3605 Before the Subcomm. on Courts, Civil Liberties and the Admin. of Justice of the H. Comm. on the Judiciary,* 98th Cong. 826 (1984) (letter from Bernarr R. Pravel, President, American Intellectual Property Law Association) ("Section 202 is intended to reverse the April 23, 1984, decision of the Court of Appeals for the Federal Circuit in *Roche Products, Inc. v. Bolar Pharmaceutical Co.,* 733 F.2d 858 (Fed.Cir. 1984)."); Memorandum from Congressional Research Service, The Library of Congress, American Law Division to House Judiciary Committee, *located at* H.R.Rep. No. 98–857, pt. 2, at 27 n.18 (1984), 1984 U.S.C.C.A.N. 2686 at 2711.

Roche v. Bolar held that the limited pre-approval experiments to obtain FDA approval still infringed a valid patent. *See* 733 F.2d at 861 ("The district court correctly recognized that the issue in this case is narrow: does the **limited** use of a patented drug **for testing and investigation strictly related to FDA drug approval requirements during the last 6 months of the term of the patent** constitute a use which, unless licensed, the patent statute makes actionable?" (emphasis added)). In overturning *Roche v. Bolar,* § 271(e)(1) allowed pharmaceutical companies to conduct such experiments to obtain FDA approval. The new section enabled those companies to begin the approval process while the patent is still in force, so that they can obtain FDA approval and begin selling immediately *after* the patent's life. Otherwise, the safety testing processes would have to wait until after the patent's life ends thus creating a lag in time when the patent would not be in force yet the companies could not enter the market pending FDA approval:

> **In order to complete this application** the generic manufacturer must conduct certain drug tests. In order to

> facilitate this type of testing, section 202 of the bill creates general exception to the rules of patent infringement. Thus, a generic manufacturer may obtain a supply of a patented drug product during the life of the patent and conduct tests using that product **if the purpose of those tests is to submit an application to FDA for approval.**

130 CONG. REC. 23060 (1984) (statement of Rep. Robert W. Kastenmeier, Chairman of the Subcommittee on Courts, Civil Liberties and the Administration of Justice, Committee on the Judiciary) (emphases added).

The Pharmaceutical Manufacturers Association echoed the Chairman's analysis of the purpose of the bill:

> The sponsors and supporters of the legislation have agreed from the beginning that generic products should not be approved for marketing prior to the expiration of a valid patent as extended under the legislation. In return, there has been a compromise agreement that **pre-approval testing** could be conducted prior to the expiration of the patent, as extended, so that marketing could begin immediately thereafter. Therefore, the bill reverses the *Roche v. Bolar* decision to permit a generic company to "use" a patented product **for the limited purpose of completing the testing necessary for FDA approval.**

Innovation and Patent Law Reform: Hearing on H.R. 3605 Before the Subcomm. on Courts, Civil Liberties and the Admin. of Justice of the H. Comm. on the Judiciary, 98th Cong. 696 (1984) (letter from Pharmaceutical Manufacturers Association) (emphases added).

On the other side of the industry, the Generic Pharmaceutical Industry Association agreed that section 202 is only for limited pre-approval experiments:

> The purpose of the foregoing provision is to permit a generic drug manufacturer to engage in **the limited experimental activities which are necessary to obtain FDA pre-marketing approval** before a patent expires so that actual competition between the generic drug and the original drug can begin immediately after the patent covering the original drug expires. **Section 202 does not authorize any activity which would deprive the patent owner of the sale of a single tablet during the life of a valid patent. In fact, the limited testing activity required to obtain FDA approval of a generic drug would not normally result in the use of even a single generic tablet for its therapeutic purpose during the life of a valid patent.**

Innovation and Patent Law Reform: Hearing on H.R. 3605 Before the Subcomm. on Courts, Civil Liberties and the Admin. of Justice of the H. Comm. on the Judiciary, 98th Cong. 926 (1984) (memorandum of Alfred B. Engleberg, Patent Counsel, Generic Pharmaceutical Industry Association) (emphases added).

The executive branch favored an even more limited exception than the one proposed in section 202 and enacted as § 271(e)(1). Nevertheless, it clearly understood the boundaries of section 202 to be pre-approval experimental use.

> This letter sets forth the Administration's views on H.R. 3605 ... First, section 202 of title II should be amended to permit experimental use of a drug by a non-patentee only during the period in which the affected patent is in restoration period. Existing patentees have relied upon accepted doctrine indicating

> that use of a patented invention **for the purpose of obtaining regulatory approval** infringes that patent. Upsetting expectations of this sort could only inhibit future innovation and investment, which depend upon the integrity of the patent laws.

Innovation and Patent Law Reform: Hearing on H.R. 3605 Before the Subcomm. on Courts, Civil Liberties and the Admin. of Justice of the H. Comm. on the Judiciary, 98th Cong. 812 (1984) (letter from David A. Stockman, Director, Office of Management and Budget, to Rep. Edward R. Madigan, Subcomm. on Health and the Environment, H. Comm. on Energy and Commerce) (emphasis added).

The pharmaceutical industry expressed concern about permitting trespass on exclusive rights, but this concern dissipated with promises that § 271(e)(1) only allowed "limited testing of drugs." *See* H.R.Rep. No. 98–857, pt. 2, at 29 (1984), 1984 U.S.C.C.A.N. 2686 at 2714.

> In this case the generic manufacturer is not permitted to market the patented drug during the life of the patent; all that the generic can do is test the drug **for purposes of submitting data to the FDA for approval.** Thus, the nature of the interference is ***de minimis.***

Id. at 30 (emphases added).

Specifically, § 271(e)(1) won approval because it was limited in time, quantity, and type. First, as to time, § 271(e)(1) only applies to **pre**-marketing approval. 130 CONG. REC. 23060 (1984) (statement of Rep. Robert W. Kastenmeier, Chairman of the Subcommittee on Courts, Civil Liberties and the Administration of Justice, Committee on the Judiciary) (see block quote above); *Innovation and Patent Law Reform: Hearing on H.R. 3605 Before the Subcomm. on Courts, Civil Liberties and the Admin. of Justice of the H. Comm. on the Judiciary,* 98th Cong. 696 (1984) (letter from Pharmaceutical Manufacturers Association) (see block quote above); *Innovation and Patent Law Reform: Hearing on H.R. 3605 Before the Subcomm. on Courts, Civil Liberties and the Admin. of Justice of the H. Comm. on the Judiciary,* 98th Cong. 742 (1984) (statement of Laurence H. Tribe, Professor of Law, Harvard Law School) ("Section 202, the thrust of which is to overturn *Roche v. Bolar* legislatively, so as to provide that it is *not* an infringement to make, use, or sell a patented invention for purposes 'reasonably related' to the development and submission of information to obtain FDA's **premarketing approval** to engage in the commercial manufacture, use, or sale of the drug after patent expiration") (emphasis added); *Innovation and Patent Law Reform: Hearing on H.R. 3605 Before the Subcomm. on Courts, Civil Liberties and the Admin. of Justice of the H. Comm. on the Judiciary,* 98th Cong. 926 (1984) (memorandum of Alfred B. Engleberg, Patent Counsel, Generic Pharmaceutical Industry Association) (see block quote above); Memorandum from Congressional Research Service, The Library of Congress, American Law Division to House Judiciary Committee, *located at* H.R.Rep. No. 98–857, pt. 2, at 27 n.18 (1984), 1984 U.S.C.C.A.N. 2686, 2711 n. 18 ("In § 202, Congress would provide that it is not an infringement to make, use, or sell a patented invention solely for uses reasonably related to the development and submission of information **for the purpose of obtaining FDA premarketing approval of a drug**.").

Second, as to quantity and type, § 271(e)(1) only applies to experimentation—and therefore would have limited impact on the patentee's exclusivity during

the life of the patent. H.R.Rep. No. 98–857, pt. 1, at 45–46 (1984), 1984 U.S.C.C.A.N. 2647 at 2678–2679 (see block quote above); *Innovation and Patent Law Reform: Hearing on H.R. 3605 Before the Subcomm. on Courts, Civil Liberties and the Admin. of Justice of the H. Comm. on the Judiciary,* 98th Cong. 696 (1984) (letter from Pharmaceutical Manufacturers Association) (see block quote above); *Innovation and Patent Law Reform: Hearing on H.R. 3605 Before the Subcomm. on Courts, Civil Liberties and the Admin. of Justice of the H. Comm. on the Judiciary,* 98th Cong. 742 (1984) (statement of Laurence H. Tribe, Professor of Law, Harvard Law School) (quoted above); *Innovation and Patent Law Reform: Hearing on H.R. 3605 Before the Subcomm. on Courts, Civil Liberties and the Admin. of Justice of the H. Comm. on the Judiciary,* 98th Cong. 926 (1984) (memorandum of Alfred B. Engleberg, Patent Counsel, Generic Pharmaceutical Industry Association) (see block quote above).

In particular, the authors made clear that section 271(e)(1) would not apply to commercial sales, i.e., the "infringing" product would not enter the market until *after* the patent's life. H.R.Rep. No. 98–857, pt. 1, at 45 (1984), 1984 U.S.C.C.A.N. 2647 at 2678 ("This section **does not permit the commercial sale** of a patented drug by the party using the drug to develop such information, but it does permit the commercial sale of research quantities of active ingredients to such party.") (emphasis added); *Innovation and Patent Law Reform: Hearing on H.R. 3605 Before the Subcomm. on Courts, Civil Liberties and the Admin. of Justice of the H. Comm. on the Judiciary,* 98th Cong. 932 (1984) (memorandum of Alfred B. Engleberg, Patent Counsel, Generic Pharmaceutical Industry Association) ("The **limited 'experimental use'** permitted by Section 202 does not, in any way, impinge on the exclusive right of the patent owner to make, use and sell the patented invention for all commercial purposes during the life of the patent. **The permitted experimental use would not result in competitive commercial activity until all valid patents expired.**") (emphases added).

The authors of this section (and I hesitate to add that I was present through this legislative process) did not imagine that § 271(e)(1) would allow *continuous, commercial* infringing sales during any portion of the life of the patent. As discussed below, Amphastar has already obtained FDA regulatory approval, and today this court rewrites the law to allow Amphastar to infringe Momenta's patent throughout *the entire life of Momenta's patent* and for the purpose of obtaining profits on *commercial sales* of a product that *competes with the patentee.*

Nowhere in the legislative history can this court find any suggestion that § 271(e)(1) would apply other than in the limited scenario of conducting *de minimis* experiments pre-approval (*i.e.,* to obtain FDA approval). Nowhere in the legislative history can this court find a hint that an "infringer" could continue to use its competitor's patented method in manufacture of each commercial batch for contemporaneous sale. Nowhere in the legislative history can this court find any mention of the post-approval, continuous, commercial sales allowed by this decision. Nowhere in the legislative history can this court find any suggestion that the mere maintenance or retention of information as part of a company's records is considered a submission that would trigger § 271(e)(1). In fact, this court makes no attempt to examine the legislative history of this section at all—a very telling silence.

Of course, this court proclaims that it finds no ambiguity requiring it to find out the purpose of the section it distorts. To the contrary, the Supreme Court found the statute can be ambiguous and "not plainly comprehensible." *See Eli Lilly,* 496 U.S. at 669, 110 S.Ct. 2683. Moreover the court strains to avoid ambiguity by discounting critical statutory phrases, namely "solely" and "submission."

To facilitate a post-approval, continuous, commercial use, the court discounts the word "solely." Indeed, throughout its opinion, the court cites the language of the statute yet omits the word "solely." *See* Majority Op. 1355, 1355–56, 1356, 1356–57, 1359, 1359–60. If one properly reads "solely" as the statute says, the result must be that Amphastar's activity is not within the statute. Its infringing activity is **not solely** for developing and submitting information to the FDA. Instead, Amphastar uses this method for the purpose of manufacturing a product to sell on the market in commerce.

Second, the court claims that the mere retention of records can satisfy the "submission" requirement in § 271(e)(1). By essentially stating that "submission" can mean not really submitting, this new interpretation reads this requirement out of the statute as well.

Specifically, despite the plain meaning of "submission of information" to mean the company actually submitting information to the FDA, the court interprets "submission of information" to mean the mere retention of information as part of a company's records. Majority Op. 1357 ("We think that the requirement to **maintain** records for FDA inspection satisfies the requirement that the uses be reasonably related to the development and submission of information to the FDA. Thus, we consider this information 'submitted' for purposes of the statute." (emphasis added)), 1358. Maintaining or keeping a document has the exact opposite meaning of submitting a document. In other words, "submission" means not really submitting anything—a strange construction of an "unambiguous" term.

This new interpretation would allow almost all activity by pharmaceutical companies to constitute "submission" and therefore justify a free license to trespass. The FDA can inspect records of any drug manufacturer and seller. *See* 21 U.S.C. § 374. Thus, the drug manufacturer need only make a record, which could potentially be inspected by the FDA, and then any activity could satisfy this new meaning of "submission."

Therefore, a reading of *all* the words in the statute and a reading of those words in light of their legislative history shows that § 271(e)(1) only permits a limited amount of pre-approval experiments to obtain FDA approval. Thus, the statute limits the exception to "solely for uses reasonably related to the development and submission of information under a Federal law which regulates the manufacture, use, or sale of drugs or veterinary biological products."

II.

This court has already decided the meaning of this statute in *Classen.* The *Classen* majority held "§ 271(e)(1) provides an exception to the law of infringement in order **to expedite development of information for regulatory approval** of generic counterparts of patented products. The statute does not apply to information that may be routinely reported to the FDA, long after marketing approval has been obtained." 659 F.3d at 1070 (empha-

sis added). As support, *Classen* looked to the legislative history: "The Report is replete with statements that the legislation concerns **premarketing approval** of generic drugs. The Report emphasizes that 'The information which can be developed under this provision is the type which is required **to obtain approval** of the drug.' " *Id.* at 1071 (emphases added).

Classen also looked to Supreme Court precedent, such as *Eli Lilly & Co. v. Medtronic, Inc.*, 496 U.S. 661, 110 S.Ct. 2683, 110 L.Ed.2d 605 (1990) and *Merck KGaA v. Integra Lifesciences I, Ltd.*, 545 U.S. 193, 125 S.Ct. 2372, 162 L.Ed.2d 160 (2005): "Every decision examining the statute has appreciated that § 271(e)(1) is directed to **premarketing approval** of generic counterparts before patent expiration." *Id.* at 1071 (emphasis added). In particular, *Classen* stated:

> Our colleague in dissent strays from statute and precedent, in arguing that any activity by any entity concerning any adversely patented product or method is exempted from infringement by § 271(e)(1), provided only that the information obtained is 'reasonably related to submitting *any* information under the FDCA,' [659 F.3d at 1083 (Moore, J., dissenting)] (emphasis in dissent), 'including information regarding **post-approval** uses.' *Id.* Such a massive enlargement of the statutory exemption is incorrect.

Id. at 1072 n. 4 (bold emphasis added).

Here, Amphastar uses Momenta's patented method in the manufacture of each commercial batch it sells. By definition, its use is not to obtain FDA approval. One can only market a drug that the FDA has already approved. Amphastar is not using Momenta's patented method for pre-approval, limited experimental use. It is not *pre-approval* because Amphastar has already obtained approval. *See* Appellant Br. 7 (Amphastar received FDA approval on September 19, 2011.); Majority Op. 1359 ("It also avoids the situation here, where a drug has received approval . . ."). Thus, its activity is post-approval. It is not *limited* because Amphastar uses Momenta's invention on a continuous basis in the manufacture of each commercial batch and during the life of Momenta's patent. It is not *experimental* because Amphastar uses Momenta's invention in manufacturing each commercial batch of its product for contemporaneous sale on the market (in commerce) to obtain profits and to compete with Momenta. This is a commercial use of an invention by a competitor to compete and trespass on the inventor's exclusive right. Amphastar's use is not for premarketing FDA approval and therefore *Classen* definitively holds that § 271(e)(1) does not apply here.

To come out the exact opposite way, the court first claims *Classen* did not turn on the pre-/post-approval distinction. Majority Op. 1358. Second, the court claims *Classen* merely held that § 271(e)(1) does not apply to "routine" submissions. Therefore, this court opines: "This case, however, fits well within Classen because the information submitted is necessary both to the continued approval of the ANDA and to the ability to market the generic drug. Here, the submissions are not 'routine submissions' to the FDA, but instead are submissions that are required to maintain FDA approval." Majority Op. 1358.

At the outset, this court must stretch too far to claim *Classen* did not turn on a pre-/post-approval distinction. The dissent actually helps identify the holding in *Clas-*

sen. The *Classen* dissent stated: "The majority concludes that the district court incorrectly interpreted the safe harbor of § 271(e)(1) because, according to the majority, § 271(e)(1) is limited to **pre-approval activities.** . . . Accordingly, I conclude that the safe harbor extends to all uses that are reasonably related to submitting any information under the FDCA, including information regarding **post-approval** uses. . . ." 659 F.3d at 1083 (emphases added).

Further, the parties and the amici certainly thought *Classen* turned on a pre-/post-approval distinction. *See, e.g.,* Classen's Opposition to Petition for Rehearing En Banc, at *1 ("Plaintiff–Appellant agrees with Hatch–Waxman, The United States Supreme Court and the Federal Circuit: 35 U.S.C. § 271(e)(1) applies only to **pre-market development activities,** there is no safe harbor after the commencement of commercial sales of a drug. An extension of 271(e)(1) into the **post-approval/post-commercialization period** is outside the scope of the Drug Price Competition and Patent Term Restoration Act and would present unworkable difficulties in its application.") (emphases added).

Moreover, this court in *Classen* did not at any point state that § 271(e)(1) applies to information "necessary both to the continued approval of the ANDA and to the ability to market the generic drug." Majority Op. 1358. Indeed, this post-approval, continuous, commercial use is the exact opposite of the *Classen* rule. *Classen* rested its holding on "premarketing approval," 659 F.3d at 1070, 1071, "limited amount of testing," *id.* at 1071, and "experimentation," *id.*

This decision ("post-approval studies"; "after approval"; "not restricted to pre-approval activities") cannot be genuinely reconciled with *Classen* ("pre-marketing approval"). Instead, the court in this decision uses the same language as the dissent in *Classen* ("post-approval"; "I conclude that the safe harbor extends to all uses that are reasonably related to submitting any information under the FDCA, including information regarding post-approval uses"). This decision should instead request the entire court to resolve the issue *en banc.*

The court distinguishes *Classen* by characterizing the activities in that case as not "mandated by the FDA," while the activities here are. Some context is in order. The patented method here is "mandated" only in that Momenta thus far has created and developed the only successful method by which one can show the FDA's requirement has been met. Amphastar is free to invent its own method to satisfy these requirements. Instead it chooses to trespass. Because it has not ventured to find another way to perform these tests, it is unfair to suggest that Amphastar's hands are tied. Indeed, to the extent the court is creating a new expansion of the statute that covers anything "mandated" by the FDA, this would unfairly attack inventors of the newest and most successful method. Such a method would be adopted or "mandated" by the FDA and then trigger the court's new infringement exception. Needless to say, that would be the exact opposite from a system that incentivizes creation and improvement.

III.

This court's interpretation of § 271(e)(1) would essentially render manufacturing method patents worthless. This court repeatedly states that the FDA's adoption of Momenta's patented method as a standard means that § 271(e)(1) should apply. Ma-

jority Op. 1358 ("the information here is not generated voluntarily by the manufacturer but is generated by FDA requirements the manufacturer is obligated under penalty of law to follow"), 1359 ("that act of infringement is, in effect, required by the federal government as part of the continuing safety and efficacy monitoring of an approved drug"), *id.* ("where a drug has received approval, but is nevertheless kept from the market based on an FDA mandated testing requirement"), 1359 ("the fact that Amphastar's testing is carried out to 'satisfy the FDA's requirements' means it falls within the scope of the safe harbor, even though the activity is carried out after approval"), 1360 ("Amphastar's allegedly infringing activities are clearly carried out according to the dictates of the Federal Food, Drug, and Cosmetic Act").

In essence, this reasoning repeals the incentives and protections of the patent act in this area. A patentee invents the first and (at the time) best method. Because of the success and utility of the inventive method, the FDA adopts that method as a standard. Because that method is "required by the FDA," this court would permit copiers to infringe. What incentive remains to invest in inventing a better test? Imagine a teacher who rewards the top student by allowing her peers to copy her exam answers. Needless to say, this approach does violence to patent law and future research incentives in this field.

And what happens if a second, less effective (patented) method appears? Will copiers be allowed to infringe that method, too? Or, instead, because it is not as good and the FDA does not adopt it as the standard, then the court's new interpretation of § 271(e)(1) does not apply and copiers can infringe the first, best method but not the second, less effective method?

IV.

The Supreme Court cases *Eli Lilly & Co. v. Medtronic, Inc.*, 496 U.S. 661, 110 S.Ct. 2683, 110 L.Ed.2d 605 (1990) and *Merck KGaA v. Integra Lifesciences I, Ltd.*, 545 U.S. 193, 125 S.Ct. 2372, 162 L.Ed.2d 160 (2005) support the holding in *Classen* and do not support this decision. Both holdings in *Eli Lilly* and *Merck* dealt with *pre-approval* activity and submissions, meaning *before* obtaining FDA approval. Further, neither even suggested that the mere maintenance or retention of information as part of a company's records could be a "submission" to the FDA. Nevertheless, the court takes phrases from those opinions out of context to allege that its new interpretation of § 271(e)(1) is consistent with those cases.

In *Eli Lilly*, the Supreme Court addressed whether § 271(e)(1) applies to medical devices in addition to drugs. 496 U.S. at 663–64, 110 S.Ct. 2683 ("This case presents the question whether 35 U.S.C. § 271(e)(1) renders activities that would otherwise constitute patent infringement noninfringing if they are undertaken for the purpose of developing and submitting to the Food and Drug Administration (FDA) information necessary to obtain marketing approval for a medical device under § 515 of the Federal Food, Drug, and Cosmetic Act (FDCA), 90 Stat. 552, 21 U.S.C. § 360e.").

The Supreme Court described how §§ 201 and 202 should be read together. Section 201 concerns activity in the early years of a patent's life. Section 202 concerns the latter years. Each section is a reciprocal counter to the other. Importantly, Congress intended the sections to deal with "premarket regulatory approval":

> The parties agree that the 1984 Act was designed to respond to two unintended

distortions of the 17–year patent term produced by the requirement that certain products must receive **premarket regulatory approval.** First, the holder of a patent relating to such products would as a practical matter not be able to reap any financial rewards during the early years of the term.... Thus, if the discovery relates to a product that cannot be marketed without substantial testing and regulatory approval, the "clock" on his patent term will be running even though he is not yet able to derive any profit from the invention. The second distortion occurred at the other end of the patent term. In 1984, the Court of Appeals for the Federal Circuit decided that the manufacture, use, or sale of a patented invention during the term of the patent constituted an act of infringement, see § 271(a), even if it was for the sole purpose of conducting tests and developing information necessary **to apply for regulatory approval.** *See Roche Products, Inc. v. Bolar Pharmaceutical Co.,* 733 F.2d 858 (Fed.Cir. 1984). Since that activity could not be commenced by those who planned to compete with the patentee until expiration of the entire patent term, the patentee's de facto monopoly would continue for an often substantial period until regulatory approval was obtained. In other words, the combined effect of the patent law and the **premarket regulatory approval** requirement was to create an effective extension of the patent term.

496 U.S. at 669–670, 110 S.Ct. 2683 (emphases added). Therefore:

> "The 1984 Act sought to eliminate this distortion from both ends of the patent period. Section 201 of the Act established a patent-term extension for patents relating to certain products that were subject to lengthy regulatory delays and could not be marketed prior to regulatory approval.... Section 201 provides that patents relating to these products can be extended up to five years ... The distortion at the other end of the patent period was addressed by § 202 of the Act.... This allows competitors, prior to the expiration of a patent, to engage in otherwise infringing activities necessary **to obtain regulatory approval.**"

Id. at 670–71, 110 S.Ct. 2683 (emphasis added).

The 1984 Act enacted the two sections to create a balance. The Supreme Court rejected the party's attempt to create a "disequilibrium" between the two sections. *Id.* at 672, 110 S.Ct. 2683.

This court's new interpretation in this case would apply the disadvantage of § 202 to a patentee who would not be able to obtain the benefits of § 201. The patentee of a manufacturing patent does not obtain the patent extension created in § 201, yet this court's new expansion of § 202 would allow its competitors to infringe during the life of its patent. The Supreme Court rejected this sort of disequilibrium. *See Proveris Scientific Corp. v. Innovasystems, Inc.,* 536 F.3d 1256 (Fed.Cir.2008) (relying on *Merck* to hold that § 271(e)(1) does not apply to infringement of patented product not eligible to obtain patent extension).

This court's new interpretation does not reserve § 202 for the "end of the patent term." Instead, its interpretation allows infringing activity continuously throughout the life of the patent, including the "early years" reserved for § 201. If, as the court claims, § 202 was meant to cover the continuous, commercial use throughout the

life of the patent, there would be no balance between § 201 and § 202. This decision improperly cuts short the life of Momenta's patent.

And, as already discussed, this new interpretation expands beyond "premarket regulatory approval." *See* 496 U.S. at 669–670, 110 S.Ct. 2683. Its interpretation allows infringing activity after the product has already been approved for sale on the market.

Surprisingly, the court claims that its "analysis is not groundbreaking: the Supreme Court came to essentially the same conclusion in 1990" and cites *Eli Lilly.* Majority Op. 1355–56. It has been quoted that "Words are easy, like the wind." Saying that something "is not groundbreaking" does not make it so.

Nowhere in *Eli Lilly* does the Supreme Court come to "essentially the same conclusion" as the majority here. The Supreme Court does not say that the mere maintenance or retention of records—with no intention to submit to the FDA but that only could potentially be viewed by the FDA if the FDA requested it—would satisfy as a "submission" to the FDA. The Supreme Court does not sanction post-approval activity. The Supreme Court does not read the word "solely" out from the statute.

It is more telling what the court's reasoning omits than what it cites. The court only relies on a single sentence from *Eli Lilly,* which it quotes out of context. 496 U.S. at 666, 110 S.Ct. 2683 ("But the phrase 'a Federal law which regulates the manufacture, use, or sale of drugs' more naturally summons up the image of an entire statutory scheme of regulation."). The Supreme Court was not even referencing the same phrase that is at issue here: "a Federal law," not "submission." In fact, that sentence is not even in the section in the Supreme Court's opinion that discusses the basis on which the Court decided the case. Instead, that sentence is in a prior section discussing the text of the statute, which the Supreme Court found "somewhat more naturally reads as the Court of Appeals determined, but that is not plainly comprehensible on anyone's view." *Id.*

The sentence cited by this court is not even a definitive holding of the Supreme Court but instead a discussion of the parties' arguments. In looking at the paragraphs following the one cited by this decision, the Supreme Court states the case for the opposing side: "On the other side of the ledger, however, one must admit that while the provision more naturally means what respondent suggests, it is somewhat difficult to understand why anyone would want it to mean that. Why should the touchstone of noninfringement be whether the use is related to the development and submission of information under a provision that happens to be included within an Act that, in any of its provisions, not necessarily the one at issue, regulates drugs?" *Id.* at 668, 110 S.Ct. 2683. On other occasions, the Federal Circuit advises against this type of slanted wordsmithing.

In *Merck,* the Supreme Court addressed whether § 271(e)(1) applies to research intended for submission for FDA approval but ultimately not submitted to the FDA. 545 U.S. at 195, 125 S.Ct. 2372 ("This case presents the question whether uses of patented inventions in preclinical research, the results of which are not ultimately included in a submission to the Food and Drug Administration (FDA), are exempted from infringement by 35 U.S.C.

§ 271(e)(1)."). In other words, the case presented an instance of limited experiments performed in the pre-approval stage of drug development.

Nowhere does *Merck* suggest that post-approval, commercial, continuous infringing use would be permitted. Indeed, *Merck* clearly lays out that § 271(e)(1) is intended for pre-approval, experimental, limited use.

> "Basic scientific research on a particular compound, performed without the intent to develop a particular drug or a reasonable belief that the compound will cause the sort of physiological effect the researcher intends to induce, is surely not 'reasonably related to the development and submission of information' to the FDA. It does not follow from this, however, that § 271(e)(1)'s exemption from infringement categorically excludes either (1) **experimentation** on drugs that are not ultimately the subject of an FDA submission or (2) use of patented compounds in **experiments** that are not ultimately submitted to the FDA. Under certain conditions, we think the exemption is sufficiently broad to protect the use of patented compounds in both situations." [545 U.S. at 205–06, 125 S.Ct. 2372 (emphasis added)] "Moreover, many of the uncertainties that exist with respect to **the selection of a specific drug** exist as well with respect to the decision of **what research to include** in an IND or NDA. As a District Court has observed, '[I]t will not always be clear to parties **setting out to seek FDA approval** for their new product exactly which kinds of information, and in what quantities, it will take **to win that agency's approval.**' *Intermedics, Inc. v. Ventritex, Inc.*, 775 F.Supp. 1269, 1280 (N.D.Cal.1991), aff'd, 991 F.2d 808 (Fed. Cir.1993). This is especially true at the preclinical stage of drug approval."

545 U.S. at 207, 125 S.Ct. 2372 (emphases added).

This court relies on some text from *Merck* that appears superficially to suggest an expansive interpretation of § 271(e)(1). But, read in context, that language has another meaning entirely. This language appears to suggest that § 271(e)(1) covers any sort of information or submission. But, this language actually appears in the context of the issue in *Merck* of whether information *intended* for submission to the FDA for approval should be covered when the information was ultimately not submitted because the drug candidate in that case lacked potential. This context is apparent in the sentences *next to* the sentence quoted by the majority, which state:

> We decline to read the "reasonable relation" requirement so narrowly as to render **§ 271(e)(*l*)'s stated protection of activities leading to FDA approval** for all drugs illusory. Properly construed, **§ 271(e)(1) leaves adequate space for experimentation and failure on the road to regulatory approval:** At least where a drug-maker has a reasonable basis for believing that a patented compound may work, through a particular biological process, to produce a particular physiological effect, and uses the compound in research that, if successful, would be appropriate to include in a submission to the FDA, that use is "reasonably related" to the "development and submission of information under . . . Federal law." § 271(e)(1).

Id. at 207, 125 S.Ct. 2372 (emphases added).

Merck does not reduce the importance of the limitation that § 271(e)(1) is re-

served "**solely** for uses reasonably related to the development and submission of information." Holding that preclinical research reasonably expected to generate information for regulatory approval does not fall outside § 271(e)(1) simply because the research fails and does not result in a regulatory application, *id.* at 206–07, 125 S.Ct. 2372, is a far cry from permitting infringement during manufacture of a commercial product merely because the infringing act also generates information that might someday be submitted to the FDA, long after marketing approval is granted. Here, Amphastar's use of the patented method is primarily for production of a commercial product; it is not "solely for uses reasonably related to" development of information.

As another point, this court claims that "the Court explicitly rejected the notion that § 271(e)(1) was limited 'to the activities necessary to seek approval of a generic drug.' " Majority Op. 1356. But, it is important to understand what *Merck* was trying to distinguish. Read in context, that phrase is referring to allowing § 271(e)(1) to include *pre-approval* activities for a *branded drug.* It was **not** stating that § 271(e)(1) included *post-approval* activities for a *generic drug.* In other words, the Supreme Court was emphasizing the words "generic drug," not the words "necessary to seek approval." Imagine ordering a computer and stating that "I do not want it delivered to my house on Wednesday." Then, the post office delivered it to your neighbor's house on Thursday. Obviously, you meant to emphasize "Wednesday," not "my house." Similarly, this court must read the Supreme Court's cases as a whole and in context.

Just because *Merck* held that § 271(e)(1) could cover pre-approval activities for not only the ANDA but also the NDA and IND, does not mean that the mere retention of documents as part of a company's records could be considered a "submission" to the FDA. In other words, if a house owner allows a hired painter to paint his house *any* and *all* shades of brown, that is not permission to choose neon orange or turquoise.

Thus, while *Merck* said that as long as an activity was *intended* for submission to obtain approval, then § 271(e)(1) applies even if the information is not actually submitted (because it is difficult to predict which drug candidates ultimately will be successful), it did not say that § 271(e)(1) applies even if the activity was *never intended to obtain approval at all.* Or if the information was *not even intended for submission to the FDA.* This court's interpretation (that the mere retention of information as part of a company's records can be a "submission" to the FDA) is indeed "groundbreaking" and the Supreme Court did not "come to essentially the same conclusion."

V.

The safe harbor provision at issue in this case, due to its origin and purpose in reversing *Roche v. Bolar,* receives attention as an exception that permits experimentation. This link to experimentation and its role in advancing the progress of technology requires some commentary as well. Too often patent law is misunderstood as impeding more than promoting innovation. This academic proposition, called the tragedy of the Anti-commons in some scholarly presentations, suggests that exclusive rights impede the flow of information and limit experimentation that might lead to the next generation of technological advance. Michael A. Heller & Rebecca S.

Eisenberg, *Can Patents Deter Innovation? The Anticommons in Biomedical Research*, 280 SCIENCE 698 (1998).

In the first place, in an era of empirical research, one might ask the reason that this academic notion has never actually been verified. Although studied, no research has substantiated this alleged attack on the patent system. In fact, "the effects predicted by the anti-commons hypothesis are not borne out in the available data." Timothy Caulfield, *Human Gene Patents: Proof of Problems?*, 84 Chi.-Kent L.Rev. 133, 137 (2009); *see also* American Association for the Advancement of Science, INTERNATIONAL INTELLECTUAL PROPERTY EXPERIENCES: A REPORT OF FOUR COUNTRIES 12 (2007) (finding the results of a 2006 survey of U.S. and Japanese researchers "offer very little evidence of an 'anticommons problem' " and that "IP-protected technologies remain relatively accessible to the broad scientific community"). Surveys of academic researchers have revealed that "only 1 percent ... report having to delay a project, and none abandoned a project due to others' patents." Wesley M. Cohen & John P. Walsh, *Real Impediments to Academic Biomedical Re search, in* 8 INNOVATION POLICY AND THE ECONOMY 1, 10–11 (Adam B. Jaffe, Josh Lerner, & Scott Stern eds. 2008), *available at* http://www.nber.org/m̃arschke/mice/Papers/cohenwalsh.pdf (citing John P. Walsh et al., *The View from the Bench: Patents, Material Transfers and Biomedical Research*, 309 SCIENCE 2002 (2005)). In other words, patents on research tools and biomedical innovations do not significantly slow the pace of research and do not deter researchers from pursuing promising projects.

The reason that patents have not been proven to impede more than stimulate technological advance is simple: it does not happen. It does not happen for several reasons. First, experiments advancing technology rarely, if ever, generate commercial value. Thus patent owners have little, if any, incentive to license or inhibit research. Stated otherwise, even if a patent owner wanted to sue or license potential researchers, experiments do not produce income or a source of damages. *See id.* at 12.

Second, in the modern age of technology, the character of technological advance has changed. The era when the Bell Labs or some other tech center could hire the most promising engineers and essentially invent everything for the world has passed. With the vast specialization of all fields of research, advances in technology require great cooperation. A new product or a new direction in biotechnology or electronics will be produced by cooperation between a professor in Chengdu, China, a young programmer in Bangaluru, India, an engineer at a large corporation in Munich, Germany, a graduate student at Tokyo University, and a team at a small start-up company in Silicon Valley. The patent system can help inform each of them of the other and bring together their incremental advances to achieve the next generation of progress in some tiny corner of human progress.

Thus, patents properly remain a tool for research and experimentation because the system encourages publication and sharing of research results. Disclosure of how to make and use the invention is the "quid pro quo" of the patent grant. *See JEM Ag Supply, Inc. v. Pioneer Hi–Bred Int'l, Inc.*, 534 U.S. 124, 142, 122 S.Ct. 593, 151 L.Ed.2d 508 (2001). In exchange for disclosure, the inventor receives a limited term of exclusivity to benefit from com-

mercialization of his invention. Without this promise of exclusivity, researchers at corporations would be forced to turn to secrecy as the best protection for their inventions. Even academic researchers may delay publication of results in order to maintain an edge over the competition, Cohen & Walsh, *supra* at 14, and the race to the patent office helps counteract this tendency toward secrecy by rewarding earlier disclosure. "The information in patents is added to the store of knowledge with the publication/issuance of the patent.... [It] is not insulated from analysis, study, and experimentation for the twenty years until patent expiration." *Classen*, 659 F.3d at 1072. Rather, information shared through patent applications is immediately available for others to build upon. It speeds the progress of scientific endeavor. In other words, the patent system's modern benefits facilitate experimentation far more than any hypothetical inhibition.

VI.

Every day, Amphastar, a competitor of Momenta, is infringing Momenta's patent. This decision allows that trespass. Moreover, to reach that result, this court must ignore its own prior decision in *Classen* and the purpose of the statute explained in the legislative history. Sadly this decision abrogates Momenta's hard-achieved property right and reallocates that entitlement to its competitors—a sad day for property owners and an undeserved victory for those who decline to invest in the expense and difficulty of discovery and invention.

LENS.COM, INC., Appellant,

v.

1–800 CONTACTS, INC., Appellee.

No. 2011–1258.

United States Court of Appeals, Federal Circuit.

Aug. 3, 2012.

Background: Online retailer of contact lenses brought cancellation proceeding against holder of registration for mark LENS, alleging holder fraudulently obtained mark, or alternatively, abandoned mark. The Trademark Trial and Appeal Board (TTAB), 2010 WL 2191900, granted competitor's motion for summary judgment of abandonment and ordered cancellation of mark. Holder appealed.

Holdings: The Court of Appeals, Linn, Circuit Judge, held that:

(1) software associated with holder's website was conduit through which retailer rendered online services, rather than good for "use in commerce" within meaning of Lanham Act, and

(2) TTAB properly relied on entire application file in ordering cancellation of mark.

Affirmed.

1. Federal Civil Procedure ⇐2470, 2470.4

Summary judgment is appropriate where the movant has established that there is no genuine issue as to any material fact and that the movant is entitled to judgment as a matter of law.

2. Trademarks ⇐1322

Federal Circuit reviews the Trademark Trial and Appeal Board's (TTAB)

CERTIFICATE OF SERVICE

I hereby certify that I electronically filed the foregoing with the Clerk of the Court for the United States Court of Appeals for the Federal Circuit by using the appellate CM/ECF system on June 27, 2014.

I certify that all participants in the case are registered CM/ECF users and that service will be accomplished by the appellate CM/ECF system.

Dated: June 27, 2014 s/ Deanne E. Maynard

CERTIFICATE OF COMPLIANCE WITH RULE 32(a)

This brief complies with the type-volume limitation of Rule 32(a) of the Federal Rules of Appellate Procedure because it contains 13,115 words.

Dated: June 27, 2014 s/ Deanne E. Maynard